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BACTERIOLOGY

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BACTERIOLOGY

FOR STUDENTS IN GENERAL AND HOUSEHOLD SCIENCE

BY

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To

JOSEPHINE HALL BUCHANAN

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PREFACE

Changes in point of view and development of the science of bacteriology have again made necessary a revision of the text. Several new chapters have been included, others completely rewritten and all revised or rearranged.

Particularly difficult in an elementary treatise is the satisfactory treatment of the subject of the classification of bacteria. An attempt has been made on the one hand to incorporate the modern point of view, and on the other not to overburden the student with difficult terminology.

There has been much revision demanded in the chapters devoted to the study of physiological characters and to the physiology of microörganisms. The chapter on enzymes and their relationships to the chemical changes produced by microörganisms has been largely rewritten and considerably expanded.

In the section relating to microörganisms and health some discussion of bacteriophage has been found advisable, likewise some revision and expansion of the treatment of immunity. The importance of the Brucella group as a cause of undulant fever in man has led to a somewhat more extended discussion.

The key to the mold genera (appendix) has been materially simplified by elimination of genera very rarely encountered in the laboratory.

The needs of students in general or liberal arts curricula have been kept in mind throughout the revision, even though there is continued emphasis on the technical phases of the subject.

R. E. Buchanan
E. D. Buchanan

Ames, Iowa
May, 1930

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BACTERIOLOGY

BACTERIOLOGY

CHAPTER I

BACTERIOLOGY AND ITS BEGINNINGS

Bacteriology is the branch of science which has to do principally with that group of microscopic plants called the *bacteria*. These have been found to be of such great practical importance that many special methods for their study and observation have been developed. These methods have also been found to be well adapted to the study of some other minute forms of life, among them such plant forms as yeasts and molds and such animal forms as certain protozoa. In consequence the study of all of these is frequently carried on in the bacteriological laboratory, and the term *bacteriology* has in consequence gradually been broadened in meaning to include consideration of all forms of life studied by bacteriological means. Practically, then, *bacteriology* is defined to include a consideration of *bacteria*, *yeasts*, *molds*, and certain *protozoa*. The word *microbiology* is frequently used in this sense, and not improbably may supplant *bacteriology* as a more general and appropriate term.

Why study Bacteriology? — The student about to begin the study of bacteriology is entitled at the inception to know some of the reasons why the subject is so frequently included in a college curriculum. It is suggested that the student early satisfy himself as to whether these arguments are reasonable and cogent, and whether the results to be anticipated are adequate and worth while.

Students are usually enrolled in general bacteriology for one or two reasons. It is urged that some knowledge of the science is of

marked advantage to any individual quite irrespective of his major vocational objective. It is a part of the background of knowledge of the educated man or woman. In addition, a knowledge of bacteriology is highly useful in many vocations; in some cases it is an essential prerequisite.

Bacteria and the related microörganisms are of major importance from the standpoint of health maintenance in man and animals. A knowledge of certain of these interrelationships may well be helpful to any individual in understanding the problems of personal hygiene and of protection from disease; it should also function as insurance against medical quackery and fraud. Many of the tasks which are of community interest and concern involve the application of bacteriological principles to prevention of disease transmission. One who has some background of bacteriological knowledge is in much better position to give intelligent coöperation to health officers and sanitarians. Some such information is desirable for the intelligent citizen in the exercise of his civic duties.

A general knowledge of the other important activities of bacteria may also be helpful in securing an understanding of many changes which are constantly to be observed in nature. Such, for example, are the phenomena termed decay, putrefaction, and fermentation. These changes enter frequently into every-day life. Some knowledge of the relationships of bacteriology to the various vocations is useful in understanding the current literature of popular science, and in understanding the interrelations and limitations of these vocations.

It is also increasingly evident that training for many specific vocations involves acquisition of special knowledge of bacteriology and its applications. The subject content of the science of bacteriology has grown so rapidly in recent years, and its vocational utilizations have been so diverse, that several important subdivisions of the subject have developed. There are numerous highly specialized applications. For example, the discoveries of bacteriologists during the past fifty years have quite revolutionized human and veterinary medicine. It is essential that the medical or the veterinary student master the elements not only of general or fundamental bacteriology, but *medical bacteriology* as well. He

should know the factors which determine the resistance of the body to disease-producing bacteria, he should be conversant with *immunology*. Some knowledge of these subjects is important not only to the physician, but likewise to all who are concerned directly or indirectly with the prevention and cure of disease. The sanitarian, sanitary engineer and public health official make practical use of the information supplied by *sanitary bacteriology;* they must needs concern themselves particularly with the ways in which pathogenic (disease-producing) bacteria are disseminated and by which their distribution may be prevented. The problems of water purification and of sewage disposal are problems of elimination of bacteria on the one hand or of their destruction on the other.

Many industries are vitally concerned with the chemical changes produced by bacteria. Some of these changes are classified as fermentations; the branch of bacteriology which treats of these changes is termed *zymology* or *zymotechnique*. Applications of bacteriology to agriculture have been numerous and important. *Soil bacteriology* is concerned with the many changes in soils produced by the activity of the bacteria, modifications requisite to soil fertility and to the growth of crops. The changes resulting from the growth of bacteria in milk and its products constitute the subject matter of *dairy bacteriology*. In *household bacteriology* there is the emphasis upon the relationships of organisms to the manufacture, deterioration, and preservation of foods. The list of vocational applications might be indefinitely extended.

One of the purposes, then, of a general course in bacteriology is to lay a foundation upon which specialization in any one of the numerous fields mentioned above may be built. It should equip the student to appreciate the many contacts between bacteriology and every-day life, to help him to safeguard himself against superstition and quackery, to enable him to coöperate effectively with the agencies working in the field of community health, to make certain incidental use in his vocation, and to assist in understanding and evaluating the science and the pseudo-science of the newspaper and the popular press as this has to do with microörganisms.

Origins of the Science of Bacteriology

It is of interest to note briefly the origins of bacteriology. As a science it is comparatively recent. Practically all our knowledge of the subject has been secured since 1860, and by far the major portion since 1890. The reasons for this slow development before the middle of the nineteenth century, and its more rapid development since, are numerous; four only of the more important may be briefly considered. They are: First, the development of the compound microscope which rendered possible reasonably adequate observation of minute cells such as those of bacteria; second, the controversy over the theory of spontaneous generation; third, the development of the germ theory of fermentation and decay; and fourth, the development and acceptance of the germ theory of disease.

History and Development of the Microscope; the Discovery of Bacteria. — Conjectures as to the existence of living beings much smaller than are visible to the naked eye were made by certain philosophers and physicians of ancient Greece. Among these men there were individuals who advanced theories as to disease causation which are not unlike those included in the modern germ theory of disease. Laboratory or controlled experiments, however, were not made. In fact, it was not until the 17th century that microscopes were sufficiently perfected to permit of the observation of such organisms.

One of the first records of observation of bacteria and other microscopic forms of life was that made by Leeuwenhoek, a Dutch lens-maker, in 1683. He constructed and used a combination of lenses (as a simple microscope) powerful enough to enable him to see bacteria in the tartar from teeth and in various decaying organic substances. His drawings are sufficiently accurate to enable one familiar with the bacteria of the mouth to recognize and identify with a considerable degree of certainty some of those which he observed. Inasmuch as many of these minute microörganisms were found to be motile, he regarded them as animals and termed them *animalcules*, though we now know them to be bacteria.

During the next hundred years the compound microscope was

developed and perfected to such a point that in 1773 a Danish scientist, Müller, was enabled to make the first classification of microscopic living things. He, too, regarded most of them as animals. Some of those he observed undoubtedly were bacteria; in fact he introduced some of the terms which we still use in bacteriology, such as bacillus, vibrio, and spirillum. However, most of the forms which he described were protozoa.

About half a century later the German investigator Ehrenberg developed a very complete classification of microscopic forms. In his book on protozoa published in 1836 he included many forms which we now know to be bacteria.

The improvement of the microscope continued. Dolland in 1844 first used the principle of immersion with the lens. This later evolved into the oil immersion objective of the modern microscope so universally used in bacteriological laboratories. The sub-stage condenser of Abbé, developed in 1870, made it possible for the lens-maker to perfect objectives with higher powers of magnification. Every increase in definition and magnification added to the value and reliability of the work done with the microscope, and each such advance was marked by a better knowledge of the morphology of bacteria.

About the middle of the 19th century certain scientists suggested that the bacteria probably were to be regarded as plants and not as animals, as had been supposed up to that time. This led the German botanist Cohn (1872–1876) to work out the first reasonably complete classification of the bacteria as a distinct group of plants. The development of modern bacteriology is usually regarded as beginning with his publications. The microscope used by Cohn gave magnifications practically equivalent to those which are used now-a-days. There has been comparatively little increase within recent years in the magnification secured by the microscope. This has been due to a combination of two factors: first, higher magnifications necessitate more convex and smaller lenses with consequent increase in the difficulty of grinding and adjusting them; second, one of the optical laws developed by the physicist is to the effect that a clear view of an object cannot be secured when the object which is being examined is smaller than

one-half the wave-length of the rays of light which are used in its examination. The microscopes usually used in the bacteriological laboratory are sufficiently powerful to make visible objects as small as can be seen because of the nature of light itself. It is, therefore, probable that the magnification secured by the best lenses in use at present cannot be materially increased. It is evident that the use of light having very short wave-lengths would be an advantage. It is quite possible to use ultra-violet and other light having very short wave-lengths to secure photographs of objects which are quite too small to be seen under the microscope.

It is clear, then, that the development of bacteriology was, in part at least, a result of the development of the modern compound microscope.

Controversy over the Theory of Spontaneous Generation. — The second factor which largely influenced the development of bacteriology was the controversy over the spontaneous generation of living things, that is, the development of life *de novo*. Some of the ancient Greeks suggested that it was possible for frogs and worms to originate spontaneously from the mud of ponds and streams, and that vermin might develop from all kinds of decaying organic matter. In this crude form this idea, of course, early disappeared, but in some form it persisted down to comparatively recent times. It was not until 1668, for example, that the idea that maggots originated spontaneously in meat when it is kept under favorable environmental conditions was seriously questioned. In that year, Redi hit upon the simple expedient of tying a screen over the mouth of a jar to prevent the entrance of flies to the meat contained therein, and observed that the maggots could develop only from flies and never formed spontaneously. Other investigators established similar facts and relationships for other types of vermin, and the theory of spontaneous generation was generally abandoned until observers began to study microörganisms.

The observation that decaying organic matter generally swarmed with bacteria led to the natural assumption by some of the older students that these germs were formed *de novo* from the dead tissues. It was argued, for example, that when a decoction of some meat

or vegetable was boiled, and all living things present presumably destroyed thereby, nevertheless frequently microörganisms developed when it was allowed to stand. It was assumed, of course, that the temperature of boiling water was sufficiently high to kill all the germs present, and that their subsequent development in the fluid proved the contention of those who argued for spontaneous generation. We know at the present time that there are certain types of resistant bacteria (or their reproductive bodies called spores) which, in some cases, may resist the temperature of boiling water for a considerable period of time.

Spallanzani (1777) found that bacteria usually did not develop in food materials which had been boiled for a time and then hermetically sealed away from the air. His results were criticised by other investigators, for if correct, they seemed to disprove spontaneous generation. Since air was excluded from his flasks, his opponents contended that the presence of air was essential to spontaneous generation. This argument was met by Schulze (1836), who passed air through sulphuric acid and then into flasks partially filled with boiled decoctions. He noted that the introduction of such air did not induce the development of microörganisms. Schwann in 1837 demonstrated the same fact by passing air through hot tubes. In other words, these investigators showed that even in the presence of air spontaneous generation did not occur in the boiled food materials. However, even these demonstrations did not entirely disarm those who opposed them. It was contended that air which had been passed through the cultures after it had been heated was, as they termed it, devitalized. They believed that air was an essential to spontaneous generation because of its stimulating action on the organic matter with which it came into contact. Those who opposed this theory of spontaneous generation contended that the untreated air when introduced into these flasks caused spoilage because it carried on its dust particles the seed (spores) of microörganisms. In other words, these flasks were seeded with bacteria from the air. These experiments, therefore, did not satisfactorily settle the question as to whether or not spontaneous generation of microscopic organisms did or did not occur.

Three different series of experiments made about the middle of the 19th century finally answered all the objections of those who believed in spontaneous generation and proved quite conclusively that life, so far as is known at the present time, must come from preëxisting life of the same type, and that there is demonstrably no such thing as spontaneous generation of microörganisms. Schröder and Dusch in 1854 showed that filtration of air through sterile cotton filters quite completely removed its power of inducing the development of organisms in boiled liquids kept in flasks. It is scarcely probable that the cotton plug to such a flask could act in any other way than as a mechanical filter. It was assumed naturally that it was able to remove all floating particles from the air which gradually passed through it. This demonstration of the efficiency of the cotton filter is the basis for the universal practice in the bacteriological laboratory of sealing sterile materials in test-tubes and in flasks by means of cotton plugs. Pasteur in 1860 showed that communication might be maintained between the sterile contents of a flask and the open air by means of a long tube so bent as to minimize the chance of any floating particles of the air gaining entrance and seeding the liquid. It was to Tyndall, however, that we owe the final proof as to the causal relationship of the floating matter of the air to the development of microörganisms. He first sought for a test whereby the complete absence of floating matter in the air might be ascertained. He found that if a beam of light was passed through an opening in a box, otherwise not illuminated, it was invisible as it passed through the interior provided there were no particles floating in the air. The principle is exactly that of observing motes floating in the sunbeam which enters an otherwise darkened room. When the air in such a box has been completely freed of floating matter as a result of sedimentation or filtration, it was found that it no longer would induce the development of organisms in boiled solutions exposed to it. The cumulative effect of these three lines of effort has been to establish with a high degree of certainty that the doctrine of spontaneous generation is false, and to justify in consequence the conclusion that living organisms of all kinds must originate from preëxisting organisms of the same kind.

The controversy which waged over this theory led to numerous studies in the laboratory. In consequence many new facts were discovered with reference to microörganisms and many methods for their study were developed. When it was found that microorganisms did not originate spontaneously, it was recognized that various kinds of bacteria might be differentiated, and that each kind would reproduce itself. This, of course, is of great practical significance, for it means essentially that disease-producing germs such as typhoid bacteria can never develop except from preëxisting germs (typhoid germs) of the same character.

The Germ Theory of Fermentation and Decay. — Soon after it was discovered that many microörganisms were present in fermenting and decaying materials of all kinds, the question arose, Are these organisms fundamentally responsible for the changes going on, or are they simply attracted or possibly nourished by chemical changes which are proceeding quite independently of them? In other words, it was asked, Do the bacteria produce the changes, or do the chemical changes which occur make the conditions favorable for the development of the bacteria?

The idea that the microörganisms are incidental and not causal was upheld with great vigor by the celebrated German chemist Liebig. He so dominated the field of chemistry in the period from 1840 to 1860 that relatively few scientists had the temerity to question his conclusions. The French investigator Pasteur, however, from time to time published experimental results from which he concluded that microörganisms are the primary cause of most types of fermentations. These conclusions were strenuously opposed by Liebig and his school, who ridiculed them as puerile and quite unworthy of serious consideration. Pasteur, however, took the problems into the laboratory and continued to pile up evidence until finally he succeeded in showing quite conclusively that the alcoholic fermentation of sugars is commonly due to the presence of yeasts, the souring of milk and the transformation of wine into vinegar are due to the activity of bacteria. Since the time of this controversy the evidence has grown until we now recognize that all putrefaction, fermentation, and decay are due to the growth of microörganisms. Further, in most cases

it has been possible to isolate and study the particular organisms responsible for each of the changes. These facts aided also in getting a background for the further studies next to be noted on the causal relationships of microörganisms to disease. They also furnished much valuable and important information concerning the transformation of multitudes of organic compounds in nature, concerning the purification of water, the disposal of waste, the enrichment of soils, and the changes both desirable and undesirable brought about in food materials.

Here again it is evident that satisfactory answers to questions as to the cause and effect relationships of microörganisms with respect to fermentation, putrefaction, decay, etc., could be secured only by taking these problems into the laboratory and studying them there. Consequently our laboratory methods and technique had their inception in part in the problems worked out by the men engaged in this controversy.

The Germ Theory of Disease. — It is true that even among the philosophers of ancient Greece it had been suggested that possibly minute worms or germs of some kind floating about in the air might be responsible for the production of disease. There was, of course, no adequate proof of these suggestions, and in general they were abandoned until the work of Leeuwenhoek already mentioned made it probable that tiny microörganisms were widespread. Plenciz (1762) apparently was the first clearly to state a modern theory of the relationships of microörganisms to disease. He claimed that each disease was caused by a particular kind of germ and that these germs could be carried from one person to another through the medium of the air. However, the science of bacteriology had not developed to such a point in his day that it was possible to secure any adequate experimental proof for his theories. They were, in other words, wholly speculative. The first satisfactory demonstration of the probable causal relationship of organisms to disease was secured by Davaine in 1863. He found that the blood of animals attacked by the disease anthrax could be shown to contain rod-shaped microörganisms, and that the disease could be transmitted by transfer of such blood to a healthy individual. A similar relationship was found by Pasteur

in 1865 when he proved an exceedingly destructive silk worm disease in southern France to be due to the activities of a protozoan parasite. Somewhat later Robert Koch was able not only to confirm the earlier results of Davaine with anthrax, but to cultivate the causal organism in appropriate nutrients in the laboratory, and to reproduce the characteristic disease in cattle by the injection of so-called pure cultures. In 1882 Koch also introduced liquefiable solid media which greatly simplified the securing of pure cultures of bacteria from the mixtures in which they are generally found under natural conditions. The use of these methods was soon followed by the isolation and identification of the kinds of microörganisms causing a wide variety of diseases. The methods of staining bacteria, in order to make them more plainly visible, were also worked out. To these studies we owe the rapid advance which has occurred since about 1890 in our knowledge of the cause of diseases, and the firm establishment of the so-called *germ theory of disease*. This should not be interpreted by the student as stating that all diseases are caused by germs. On the contrary it has made possible the very clear differentiation of diseases into two groups, those caused by germs (and called in consequence *infectious*), and those not caused by germs (or *non-infectious*). To-day we are familiar with the microörganisms which cause a considerable proportion of the infectious diseases of man and animals. There are, however, some diseases which we believe to be infectious but whose causal microörganisms have not yet been detected.

The development of the germ theory of disease has quite completely revolutionized much of the practice of modern medicine. It has determined largely the direction and magnitude of growth of modern sanitary science and of preventive medicine. It is evident that when it was found that microörganisms were responsible for many diseases every effort was made to study these in the laboratory in order to determine methods of combatting these organisms and the diseases which they produced. The modern importance of bacteriology has come about, at least in part, as a result of the development of the modern germ theory of disease causation.

SECTION I

MORPHOLOGY, CLASSIFICATION, AND DISTRIBUTION OF MICROÖRGANISMS

CHAPTER II

ORGANISMS USUALLY INCLUDED IN A DISCUSSION OF BACTERIOLOGY

THERE is no single entirely suitable English word that includes all the forms of microscopic life usually recognized in the field of bacteriology. It has been previously noted that four distinct groups of organisms are to be considered, the bacteria, the yeasts, the molds, and the protozoa. Three different words are in common use for their designation. The first, *microbe*, comes from the French, and means a small or microscopic living thing. It is practically equivalent to the word *microörganism*. Sometimes the term *germ* is used as a synonym. This perhaps is satisfactory providing there is no confusion between the meaning of the word germ when used in this sense and its numerous other meanings.

It should be again noted that the first three of the groups enumerated above, the bacteria, the yeasts, and the molds, belong in the plant kingdom, and that the protozoa, on the other hand, are placed in the animal kingdom. The question at once arises as to the methods or criteria which may be used in the differentiation of plants and animals or, more concretely, the bacteria, yeasts, and molds on the one hand, and the protozoa on the other.

Why Bacteria are regarded as Plants and not Animals. — In the preceding chapter it was noted that the earlier observers of the bacteria regarded them as animal rather than as plant forms, largely because many of them were found to be able to swim about, *i.e.*, were *motile*. Later students concluded that they were plants. In order to determine why there has been this change in point of view it is necessary that the differences between plants and animals be pointed out.

It is not difficult to differentiate between the higher animals and the higher plants, since there are many points of contrast plainly to be seen. With the microscopic organisms much greater difficulty is often experienced; in fact it is possible to find forms intermediate between plants and animals such as constitute a complete series of intergradations between the two groups. This difficulty of separation of plant from animal forms is particularly pronounced with the bacteria, for they are among the simplest known plants, but intergrade with some of the simpler protozoa. The reasons for the general inclusion of bacteria among the plants may be enumerated as follows:

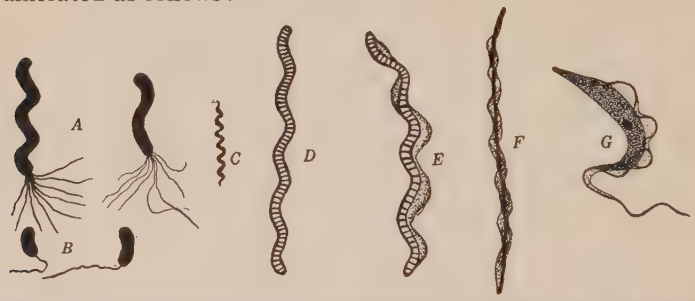


FIG. 1. — Intergradations between bacteria and protozoa. *A–B*, bacteria of genera Spirillum and Vibrio; *C–F*, Intermediate forms. *C*, Treponema; *D*, Saprospira; *E*, Cristispira; *F*, Spirochaeta; *G*, protozoan, Trypanosoma.

In most cases the cells of plants differ from those of animals in the possession of a firm and well-differentiated cell wall, quite distinct from the living cell contents or protoplasm. On the other hand the bounding surface of the animal cell is usually a more or less differentiated portion of the protoplasm itself. It is true that animal cells sometimes form a wall, as does a protozoan when it secretes a firm membrane and goes into a resting stage, that is, encysts. The regular possession of a firm wall by the cells of bacteria is one reason for grouping them with plants.

The fact that there are forms of life which are intermediate between bacteria and protozoa has sometimes led to confusion. Certain slender spiral forms in particular show marked resemblances to both bacteria and protozoa; they are placed sometimes with one and sometimes with the other. One factor which has tended to

cause these to be regarded as bacteria in recent years is that the cells have been found to multiply (like bacteria) by transverse fission, while the related protozoa multiply by longitudinal division of the cell. Fig. 1 illustrates some of the forms which are intermediate between the bacteria and protozoa.

The point is sometimes made that bacteria have cell walls which resemble those of plants in their chemical composition. This is in general true; the bacterial cell wall does resemble closely that of the fungi and certain of the algæ, though not that of higher plants.

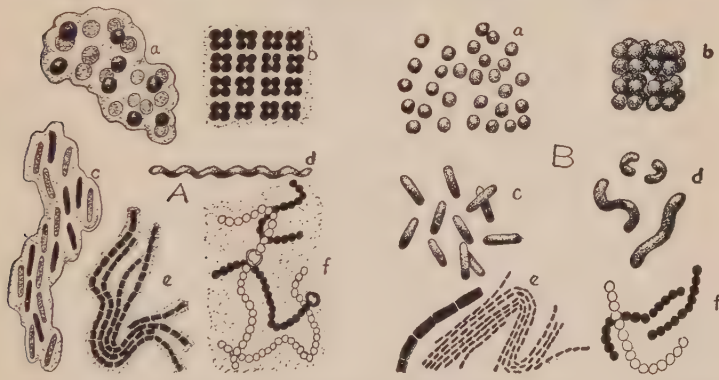


FIG. 2. — To illustrate the close relationship of the bacteria to the blue-green algæ. The figures to the left (*A*) show blue-green algæ, those to the right (*B*) bacteria. Those forms most closely resembling each other are lettered alike. *A*, blue-green algæ: *a*, Aphanocapsa; *b*, Merismopedia; *c*, Gleotheca; *d*, Spirulina; *e*, Phormidium; *f*, Nostoc. (All adapted from West.) *B*, bacteria: *a*, Micrococcus; *b*, Sarcina; *c*, Bacillus; *d*, Spirillum; *e*, Bacillus in chains; *f*, Streptococcus.

A still more cogent reason for regarding the bacteria as plants is that there are very many evidences of close relationship to other unicellular forms quite generally regarded as plants. The relationship to the blue-green algæ (Schizophyceæ) is particularly close. Fig. 2 illustrates the resemblance of these groups morphologically. In many cases it would be quite legitimate to regard the bacteria as algæ which had lost their green color (chlorophyll).

The fact that many (not all) bacteria are motile, swimming about actively in water by means of organs of locomotion (called flagella), should not be regarded in any sense as an animal charac-

teristic. We are so accustomed to seeing the higher plants anchored in place, and the higher animals moving that we are apt to associate motility only with animals. This is quite unwarranted, for many typical plant cells are known to be able to swim about, among them many of the algæ, some of the fungi, and certain of the reproductive (sperm) cells of other plants, such as mosses, ferns, and

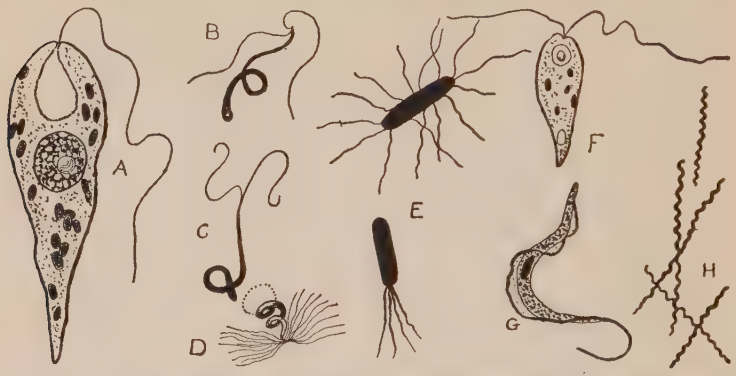


FIG. 3. — To illustrate the fact that organs of motion and motility are not exclusive animal characteristics. *A*, Euglena, a unicellular green alga; *B*, motile sperm of the Chara, one of the algæ; *C*, motile sperm of a moss; *D*, motile sperm of a fern; *E*, motile bacterial cells; *F*, a motile protozoan from stagnant water; *G*, a trypanosome; *H*, Treponema. *A*, *B*, *C*, *D*, and *E* are plants or plant cells; *F*, *G*, and *H* are protozoan cells. All of these cells are motile by means of flagella or cilia.

even of some seed plants (as the gingko or maiden-hair tree). Fig. 3 illustrates that organs of motion and motility are not exclusive animal characteristics.

Position of Bacteria, Yeasts, and Molds in the Plant Kingdom. — It was previously noted that the organisms to be considered under the heading of microbiology (or of bacteriology in a broad sense) are the bacteria, the yeasts, and the molds. Just where do these forms belong in the plant kingdom?

Plants are divided into four great groups: the *Spermatophyta* or seed plants, the *Pteridophyta* or fern plants, the *Bryophyta* or moss plants, and the *Thallophyta* or thallus plants. The last group is the one that includes the bacteria, yeasts, and molds. It is differentiated from all other groups of plants by the lack of a complex

plant body. Sometimes the plant consists of but a single cell, frequently of a thread of cells united end to end, but never forming a complex plant tissue with differentiation into roots, stems, and leaves.

The group *Thallophyta* is a very large one, containing tens of thousands of species. It is usually divided into three subgroups: the *Schizophyta* or fission plants, the *Algæ*, and the *Fungi*. The members of the group *Schizophyta* are distinguished from all other plants by being unicellular, and by having no method of multiplication other than fission, or cell division. This group is divided into subgroups: the *Schizophyceæ* or blue-green algæ, and the *Schizomycetes* or bacteria. The cells of the former contain chlorophyll or "leaf green," while the cells of the bacteria are devoid of this pigment. The bacteria are to be studied, inasmuch as they contain so many forms of economic importance.

The group Algæ includes the seaweeds, pond scums, water-silks, and similar forms. None of them are considered further. The group of Fungi differs from the Algæ in the absence of chlorophyll and includes the toadstools, puffballs, mushrooms, smuts, rusts, mildews, yeasts, and molds. Only the two groups last mentioned are included in the scope of bacteriology.

The following classification may assist in getting a clear idea of the position of the plant groups to be considered. These are given in boldface type, the others in italics.

- **Thallophyta** — Simple, undifferentiated plants. Never developing roots, stems, or leaves
  - Schizophyta. Fission plants
    - *Schizophyceæ* or blue-green algæ
    - **Schizomycetes** or bacteria
  - Algæ, including seaweeds, pond scums, water-silks, etc. Contain chlorophyll
  - Fungi, without chlorophyll
    - **Yeasts**
    - **Molds**
    - *Mildews*
    - *Smuts*
    - *Rusts*
    - *Mushrooms*
    - *Puffballs*, etc.

Differentiation of Bacteria, Yeasts, and Molds. — If bacteria, yeasts, and molds are to be studied, it is necessary that methods for their differentiation should be pointed out. In general there

is little difficulty in such sharp differentiation, though occasionally intergrading or intermediate forms are encountered which prove somewhat troublesome.

The molds on the one hand are readily separated from the bacteria and the yeasts on the other. Molds are multicellular, bacteria and yeasts are unicellular. While bacterial or yeast cells may adhere in masses, groups, or chains, the individual cells are independent plants; in the mold many cells are united to form a filamentous plant body.

The differentiation of yeasts and bacteria is somewhat more difficult. The following are the more easily recognized differences:

1. In general yeast cells are somewhat larger than bacterial cells. This is not always true, but it holds for most of the bacteria and yeasts commonly studied in the laboratory. Differentiation on the basis of observation of size can result only from experience.

2. Most bacteria multiply by transverse fission (splitting) of the cell, most yeasts multiply by a process of budding. This is usually the easiest method of differentiation, and it holds good generally for the bacteria and yeasts most commonly cultivated in the laboratory. One type of yeast, however, is known which multiplies like bacteria, by fission. Furthermore, some types of bacteria, particularly in old cultures, may produce buds. The differentiation is however sufficiently accurate so that the name Schizomycetes (fission-fungi) and Blastomycetes (budding fungi) have been applied to the bacteria and yeasts respectively.

3. Bacteria usually may be differentiated from yeasts on the basis of cell structure. Bacterial cells rarely show a well-marked differentiation of the protoplasm into nucleus and cytoplasm, but such differentiation may be readily demonstrated in the yeasts.

4. A criterion which frequently proves helpful is that of the changes produced. Most (not all) of the common yeasts are active in the fermentation of sugar to alcohol to carbon dioxide. While some bacteria may produce small amounts of alcohol in their growth, in general alcoholic fermentation is due to yeasts.

Other differences between yeast and bacteria, and between these and molds will become evident as these groups are discussed, and their morphology and physiology are examined.

CHAPTER III

MORPHOLOGY OF THE BACTERIA

Shape of Bacterial Cells. — Bacterial cells are usually of one of three shapes; spheres, straight rods, or bent rods. Sometimes rods when sufficiently long are filamentous.

A spherical organism is designated as a *coccus*. Considerable variation in shape is found among the cocci, although they are typically globular. When two cocci lie side by side, the approximated sides are apt to be flattened, sometimes even concave. One species of coccus, for example, in which the cells are usually found in pairs, shows one side flattened and the other somewhat pointed. Isolated cells in most cases, however, assume the spherical shape.

A rod-shaped organism is termed a *bacillus*. The rod may be long or short; it may have rounded ends, or they may be truncate or even concave.

A spiral organism is called a *spirillum*. It may be slender or broad, short or long. A single cell may show several turns of the spiral, or may be so short as to appear as a slightly bent rod.

Filamentous bacteria are sometimes termed the *trichobacteria*.

Arrangement and Grouping of Bacterial Cells. — Bacterial cells are frequently surrounded by a thin (rarely a thick) layer of gelatinous material which tends to cause the cells to cling together. The shape of the group of cells thus formed and their arrangement within the group are determined largely by the manner of multiplication and the direction of the planes of division.

The cocci show the greatest diversity of cell arrangement. It is a general rule among bacteria that the wall that divides a cell into two daughter cells is formed at right angles to the longest axis. Since cocci have no longest axis, cell division may occur in any direction. The daughter cells may immediately separate

after the completion of the division. This results in the development of isolated cells only. With most forms, however, the cells

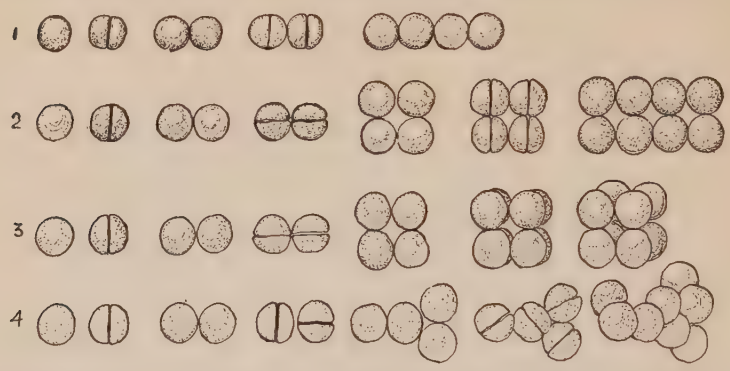


FIG. 4.— Successive steps in the formation of the various groupings of the cocci. 1, streptococcus; 2, micrococcus, with cells arranged in tetrads; 3, sarcina; 4, staphylococcus.

remain together for a time at least. A coccus in which the successive planes of division are constantly parallel forms, as a result, rows of cells resembling a chain of beads. This type is known as a *streptococcus*. In some forms the cells remain typically united

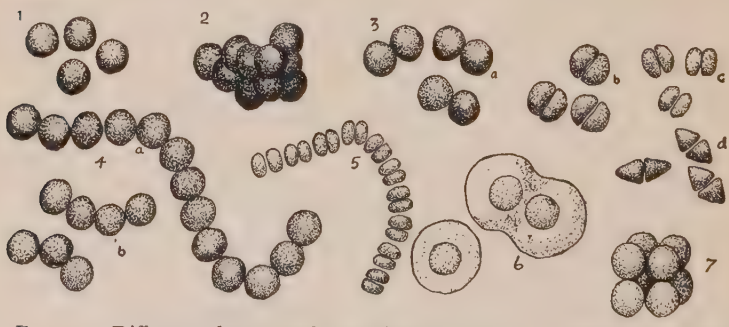


FIG. 5.— Different shapes and groupings of cocci. 1, micrococcus, with cells separate; 2, staphylococcus, with cells arranged in irregular masses or grapelike clusters; 3, diplococci, with cells in pairs; *a*, cells spherical; *b*, cells flattened; *c*, cells flattened, disk shaped; *d*, cells flattened on proximal sides, and pointed at opposite; 4, *a*, streptococcus with many elements; *b*, streptococcus with a few elements; 5, streptococcus composed of diplococci; 6, capsulated micrococcus; 7, sarcina.

in pairs; one of this type is termed a *diplococcus*. When the cells remain attached in irregular masses, resembling clusters of grapes, the term used is *staphylococcus*. This last grouping usually results from irregular division of the cells, the planes of division being at

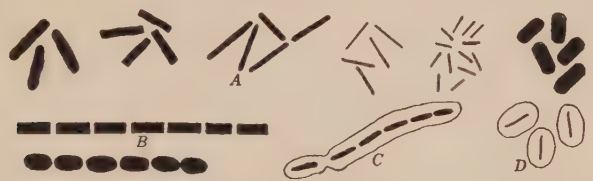


FIG. 6.— Different shapes and groupings of the bacilli. *A*, isolated bacilli; *B*, streptobacillus; *C*, capsulated streptobacillus; *D*, capsulated bacillus.

all angles to each other. A *sarcina* is a coccus which divides successively in three planes, each of the planes being perpendicular to the other two. The cells cling together in cubes, made up of eight cells or of multiples of eight. The groups are sometimes likened in appearance to miniature cotton bales. These groupings and coccus shapes are illustrated in Fig. 4 and Fig. 5.

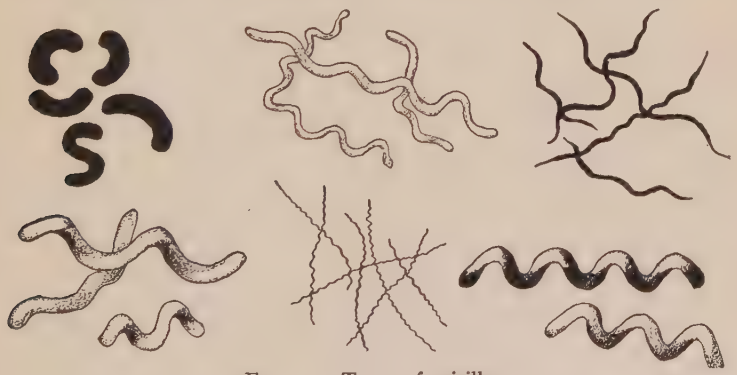


FIG. 7.— Types of spirilla.

Bacilli may separate at once after cell division, or they may remain united in chains. Such chains are sometimes termed *streptobacilli* (Fig. 6). Inasmuch as bacilli never divide longitudinally, no other grouping can occur. Spirilla are generally separate, although occasionally they may occur in chains (Fig. 7).

The trichobacteria consist of long threads which may be unbranched or branched. If the latter, the branching may be either true or false. The differences between these types is illustrated in Fig. 8.

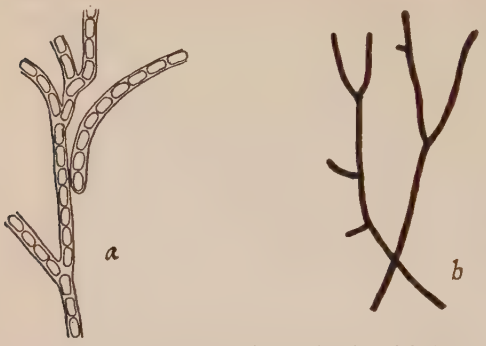


FIG. 8. — False and true branching in trichobacteria. *a*, false branching; *b*, true branching.

Under certain conditions, usually those not favorable for growth, bacteria may show abnormal or unusual shapes. These are termed *involution* forms. Certain species of bacteria show this much more readily than others. Fig. 9 illustrates certain bacteria with the commoner types of involution forms.

Size and Measurement of Bacteria. — The unit used in measurement of microscopic objects is the micron, usually abbreviated as the Greek letter μ (mu). The micron is the one one-thousandth part of a millimeter, approximately one twenty-five-thousandth of an inch.

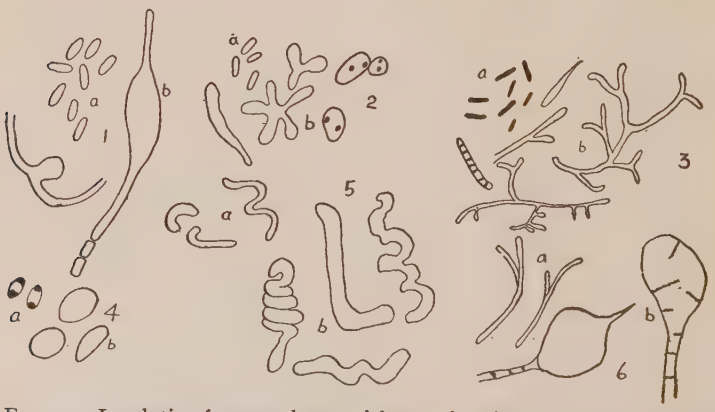


FIG. 9. — Involution forms and normal forms of various bacteria. *a*, normal forms; *b*, involution forms. 1, *Acetobacter acetosum* (adapted from Henneberg); 2, *Rhizobium leguminosarum;* 3, *Mycobacterium tuberculosis;* 4, *Pasteurella pestis;* 5, *Lactobacillus delbrucki* (adapted from Henneberg); 6, Actinomyces.

Bacteria are of many different sizes. Some kinds are so minute as to be invisible under the highest magnifications of our microscopes; a few species are large enough to be seen with a simple hand lens and low magnification. This means that some bacteria are probably less than 0.1 μ in diameter. Most of the kinds studied in the laboratory are between 0.5 μ and 5 μ in diameter.

The minuteness of these bacterial cells deserves some special emphasis. If an "average" bacterial cell has a volume of one cubic micron (1.0 μ^3), it would require one trillion (one million million) to have a total volume of one cubic centimeter, and about fifteen trillion (accurately 15,625,000,000,000) to have a volume of one cubic inch. Somewhat later we shall discuss the numbers of bacteria contained in various materials, such, for example, as souring milk. We may find perhaps a million per cubic centimeter. If the bacterial cells each had a volume of one cubic micron, only one one-millionth part of the volume of the milk would be made up of bacteria. It is not surprising, therefore, to find bacteria increasing in numbers under favorable conditions until as many as one or more billions are present per cubic centimeter.

To an organism which is ultra-microscopic it is customary to give the name *filterable virus*. This name is used to indicate the fact that some are so small that they may pass through a porcelain filter with pores so minute that the bacteria usually observed cannot pass. Such organisms may, of course, not be bacteria at all. They can be known only by the effects which they produce. Some, for example, are known to be able to produce disease.

Structure of the Bacterial Cell. — The minute size of bacterial cells renders them more difficult to study than those of the higher animals and plants. The cell structure, too, is simpler and more primitive than that of the higher forms. The subject will be discussed under four headings: *Cell wall*, *Capsules*, *Cell contents*, and *Organs of motion*.

Cell Wall of Bacteria. — The bacterial cell wall is a relatively firm membrane surrounding the living matter or cell contents. Its chemical composition is not the same in all bacteria. In a very few forms it gives the reactions characteristic of cellulose, such as coloring blue with chloriodide of zinc. In other cases,

chemical tests have shown it to contain nitrogen, and to resemble in chemical composition the chitin covering the bodies of insects. In still other cases it is probably composed of hemicelluloses, pectins or similar compounds related to the carbohydrates.

The cell wall of bacteria is to be regarded as a protective membrane. All the food taken in by the organism must pass through this membrane by diffusion, but it is probably not selective in its action as to the solutes passing through, in contrast to the protoplasmic membrane later to be considered.

Cell Contents of Bacteria. — The protoplasm or living material of the bacterial cell usually fills the interior. Its outer layer, or that portion next inside the cell wall, is differentiated as a membrane (as is the case in all plant cells) known as the *ectoplast*. This ectoplast is one of the most important structures of the cell from the standpoint of cell nutrition. In many (if not all) bacterial cells it is a semipermeable membrane; that is, it will allow some substances in solution to pass through, but not others. It determines, in other words, what substances may enter and what may leave the protoplasm. When certain bacterial cells are placed in strong sugar or salt solutions, the protoplasm shrinks, the ectoplast acting as an osmotic membrane. A cell showing shrunken protoplasm is said to be *plasmolyzed*, this phenomenon being known as *plasmolysis* (Fig. 10).

Fig. 10.— Plasmolysis of various bacterial cells.

Whether or not the bacterial cell contains a definite nucleus is even yet a matter of dispute. In certain forms granules, probably of chromatin and therefore nuclear in nature, behave during cell division something like nuclei. They probably represent a primitive and poorly developed nucleus. It should be noted that the bacteria are not alone among plants in the absence of a well-differentiated nucleus; in this they resemble certain of the blue-green algæ.

Granules and cell inclusions of various kinds are sometimes observed in bacteria, among them *metachromatic granules*, granules of *glycogen*, *sulphur granules*, and *oil globules* (Fig. 11). Meta-

chromatic granules get their name from their ability to take up the basic aniline dyes as does chromatin. They are particularly conspicuous in the diphtheria bacillus and in certain lactic acid bacteria. In the former they are especially useful in diagnosis. They have been regarded by some investigators as the result of plasmolysis in the mature and old cells, by others as granules of reserve food. They may be identified by the fact that they stain brown when treated with a solution containing iodine. *Sulphur* granules are present in certain bacteria that develop in water containing hydrogen sulphide. They may be recognized by fixing

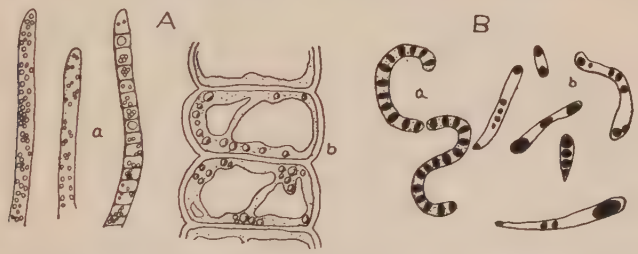


FIG. 11. — Cell inclusions of bacteria. *A*, sulphur granules: *a*, *Beggiatoa alba*; *b*, *Beggiatoa mirabilis*; *B*, metachromatic granules: *a*, *Spirillum undulum*; *b*, *Corynebacterium diphtheriæ*. (*Aa*, adapted from Winogradski; *Ab*, from Hinze; *Ba*, from Fischer; *Bb*, from Lehmann and Neumann.)

the organisms to a glass slide and treating with carbon disulphide, then allowing it to evaporate. The sulphur first dissolves and then crystallizes out in characteristic forms, which may be readily identified.

Capsules and Sheaths. — A capsule is a very much thickened, gelatinous outer portion or covering of the cell wall produced by some bacteria. A thin capsule or layer of capsular material is present in most bacteria, but in only a few does it become very thick and conspicuous. The capsule is usually partially soluble in water, or at least swells greatly in it. Capsulated organisms, (Fig. 12) therefore, when growing in suitable solutions, may make these solutions gelatinous or slimy. Such is the origin, for example, of ropy bread, and slimy milk and cream. This capsular material is in some cases nitrogenous, in others non-nitrogenous. A substance closely resembling the mucin of mucus has been identified

in some species; in others polysaccharides and related compounds, as mannans, galactans, and dextrans, have been described. An organism may form a capsule when growing under one environment but not under another. For example, the bacillus that causes anthrax produces capsules in the blood but not in most culture fluids; the organism that causes a slimy fermentation in sugar sirup forms the capsular material only in the presence of cane-sugar. Other organisms develop capsules only in milk or in urine.

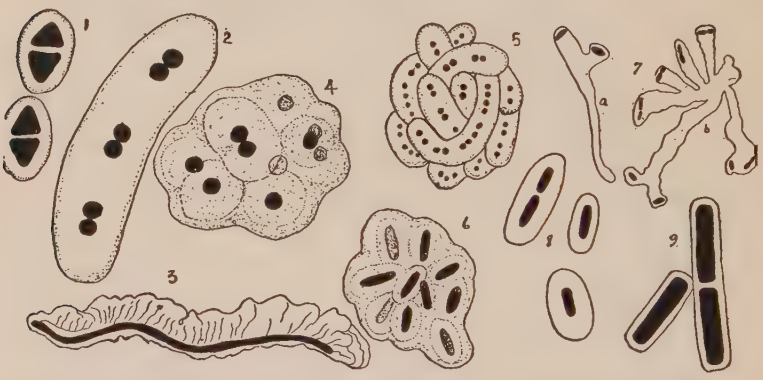


FIG. 12. — Types of capsulated bacteria. 1, *Diplococcus pneumoniæ;* 2, 4, and 5, *Leuconostoc mesenteroides*, from sugar vats (adapted from Lafar); 3, *Lactobacillus vermiformis*, from ginger beer (adapted from Ward); 6, a bacterium from a zoöglœa in sewage; 7, *Bacterium pediculatum*, showing the unilateral development of the capsular material (adapted from Koch and Hosæus); 8, *Bacterium lactis viscosum*, from slimy milk; 9, *Bacillus anthracis*, from blood.

The capsule is not always readily demonstrated in a microscopic examination. Special staining methods are frequently necessary.

In some cases the capsular material of cells forms a gelatinous mass in which the bacterial cells appear to be embedded. Such a collection of organisms together with the surrounding gelatinoid envelop is termed a *zoöglœa*. In sewage and other highly contaminated water, in decomposing foods, etc., such zoöglœæ are not uncommon (Fig. 13.)

Bacteria growing in chains sometimes secrete a firm membrane about the whole filament, forming a tube in which the organisms live. Such a membrane is termed a *sheath* (Fig. 14). Various

substances such as compounds of iron or calcium may be deposited in this sheath.

Organs of Motion. — Many species of bacteria have the power of independent movement when in suspension in a suitable liquid. This motility is due to the presence on the bacterial cells of one

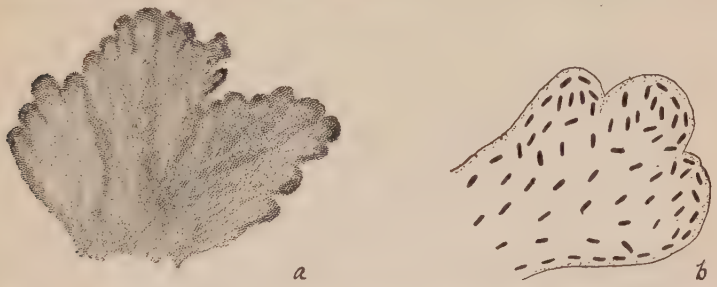


FIG. 13. — Zoöglœæ. *a*, a zoöglœa as it appears when slightly magnified; *b*, margin of a zoöglœal mass highly magnified, showing the individual bacteria.

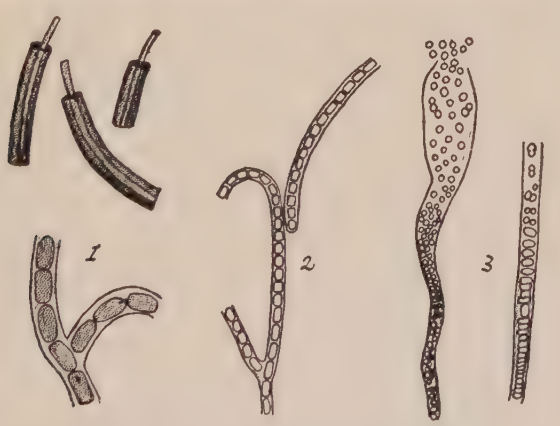


FIG. 14. — Bacterial sheaths. 1, Chlamydothrix from water containing iron; 2, *Cladothrix dichotoma;* 3, *Crenothrix polyspora.* (1 and 3 adapted from Fischer.)

or more delicate, hairlike appendages termed *flagella* or *whips.* The flagella move actively in the water, propelling the organism. There is some debate as to the exact origin of these flagella, a few investigators claiming that they are outgrowths of the gelatinous layer external to the cell wall or of the wall itself; the great ma-

jority, however, believe that they are slender outgrowths through the cell wall from the protoplasm within. They are very delicate, easily break off, and disintegrate, and are difficult to demonstrate. They are so slender that except with large organisms and with certain lighting the flagella cannot be seen in unstained mounts. Special methods of staining are necessary for their satisfactory demonstration. Few species of cocci, many species of bacilli, and most of the species of spirilla possess flagella and are motile.

The number and location of the flagella on the organism permit a separation of bacteria into groups dependent upon the type

FIG. 15. — The flagella of bacteria. *A*, Atrichous (non-flagellate) bacilli; *B*, Monotrichous bacteria; *C*, Lophotrichous cells; *D*, Amphitrichous cells; *E*, Peritrichous cells.

of flagellation (Fig. 15). An organism with a single flagellum at one end of the cell (polar) is *monotrichous;* one with a cluster of two or more flagella at one end is *lophotrichous;* one with a cluster at each pole *amphitrichous*, and one with flagella on all sides *peritrichous*. Some authorities do not differentiate between lophotrichous and amphitrichous bacteria, believing the latter to be merely a preliminary growth phase of the former.

A few bacteria, particularly those previously noted as intermediate between the true bacteria and the protozoa, do not possess flagella, but are nevertheless motile. For the most part these organisms are very slender and relatively long. They swim in much the same fashion as a snake or an eel, by a sinuous bending of the cell body, that is, they are flexuous. It is this type of

motility which is argued as evidence of their protozoan (animal) affiliations.

Reproduction of Bacteria. — All bacteria multiply by fission, that is, by a transverse splitting of the cell into two. This is frequently termed *vegetative reproduction*. In addition certain bacteria produce various types of reproductive bodies, in some cases formed by a process of budding from the cell surface, in other cases resulting from internal segmentation. In young, rapidly growing cultures of bacteria fission is the normal method of reproduction, in older cultures other methods may be found.

Some bacteria produce specially resistant cells fitted to *reproduce* the species after a period unfavorable for growth. These cells are termed *spores*.

FIG. 16. — Vegetative reproduction or cell division of a bacillus (*Bacillus oxalaticus*). Note the gradual elongation of the cell, and the development of the cross walls. One cell division is not always complete before a second is begun. (Adapted from Migula.)

The apparent variety of methods of cell reproduction among bacteria, and the variability of the morphology of the cells in cultures as they become older has led some investigators to conclude that bacteria in some cases, at least, may have a relatively complex life history. Others have urged that there is in some cases evidence of sexual reproduction. While it is quite probable that the life history of bacteria is not as simple as has been frequently supposed, much of the evidence of complex life cycles is conflicting, and will not be reviewed here.

Vegetative Multiplication and Reproduction. — The usual method of cell multiplication in actively growing cells is by the formation of a transverse cell wall, and the two halves then split apart, becoming independent even though still held together by a gelatinous covering (Fig. 16). The plane of this division is always at right

angles to the longest axis of the cell. This division of cells may occur with considerable rapidity, a short time being required for the organism to grow to full size and split into two cells. Several common species of bacteria may reach maturity and divide in half an hour, in some cases, even in eighteen to twenty minutes. Under favorable conditions of temperature and food supply this rate may be maintained for a considerable period; but the gradual accumulation of the waste products of growth or the using up of the available food finally diminishes this rate. An organism multiplying in this geometric ratio in the course of two days would have a progeny represented by a number having twenty-nine figures. In a few days, such multiplication would result in the formation of a mass of bacteria equal in bulk to the earth itself. Conditions, of course, are never long enough favorable for such indefinite increase. This rapidity of multiplication is of the greatest economic importance, however, for it enables bacterial cells, in spite of the slight bulk of a single organism, to produce very considerable chemical changes in a short time, as in fermentation. Milk, for example, with an initial seeding of a very few bacteria capable of producing lactic acid, if allowed to stand in a warm place, will sour within a few hours.

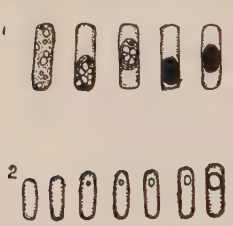


FIG. 17. — Development of bacterial spores. 1, development of spores of *Bacillus anthracis;* 2, development of spores of *Bacillus subtilis*. (1, adapted from Fischer; 2, adapted from Migula.)

Spore Production. — A spore is a cell set apart for reproduction. It is usually more resistant to unfavorable environment than the vegetative cell from which it developed. Spores are produced by practically all plants, even the highest, and by some forms among the protozoa. Spores are produced by all three groups of plants here considered: bacteria, yeasts, and molds.

The spore produced by certain kinds of bacteria is known as an *endospore* because it is always formed within the bacterial cell. Not all bacteria form endospores. None of the cocci are known to produce spores. Many of the bacilli and possibly a few spirilla are spore-forming. The endospore formation in a bacterial cell is first indicated by the appearance within the cell of highly refrac-

tile granules. These tend to collect at one point in the cell and unite to form a larger globule. This increases in size and density and finally surrounds itself with a firm membrane or spore wall (Fig. 17). The spore seems to be made by the contraction of the protoplasm with extrusion of water. A cell containing a spore is sometimes termed a *sporangium*. Usually only a single spore develops within a bacterial cell; occasionally two are found, one at either end. The spore is usually ellipsoidal or oval in shape, rarely spherical.

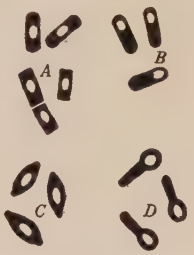


FIG. 18.— Bacterial endospores. *A*, equatorial spores of *Bacillus subtilis; B*, polar spores; *C*, spores of a clostridium; *D*, plectridiam.

The position of the endospore within the cell and its diameter relative to that of the cell are of considerable assistance in the separation and identification of certain species of bacteria. Spores may be either *equatorial* or *polar;* that is, situated at the center or near the end of the cell. The spore may have a diameter less than that of the mother cell, in which case the shape of the cell is usually unaltered, or it may be greater and cause a distortion in shape. A large equatorial spore results in the formation of a spindle-shaped sporangium, usually termed a *clostridium* (Fig. 18). Such a spore at the pole gives a drumstick type of sporangium (a *plectridium*), such as is found in the organism causing lockjaw or tetanus.

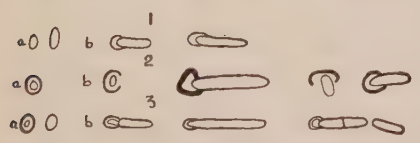


FIG. 19. — Germination of bacterial spores. 1, *Bacillus parvus;* 2, *Bacillus sphæricus;* 3, *Bacillus alvei.* (Adapted from Neide.)

At maturity, the old cell or sporangial wall generally disintegrates, liberating the endospore. In a few species the wall is persistent. A spore may *germinate* when it comes under favorable conditions for growth. Germination results in the formation of a new rod. This may be accomplished simply by a swelling of the spore contents and a stretching of the membrane, or by its rupture. If the latter, the break may occur at one or both poles, or at one side (Fig. 19).

Since in most species a single spore develops from a single bacterial cell, it is evident that sporulation among the bacteria cannot be regarded as a method of multiplication. It should be considered simply as a means of reproduction. Later it will be shown that among the yeasts and molds spore production is not only a means of reproduction but of multiplication as well.

It was noted that certain bacteria may produce reproductive bodies or cells that are not endospores. In some cases these apparently develop from the cell by a process of budding, in others

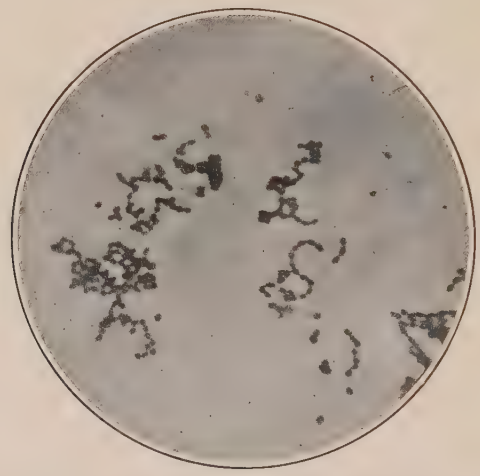


FIG. 20. — Conidia of *Actinomyces*, occurring in chains resembling streptococci. These chains project above the surface of the medium. (× 1000.)

by a process of segmentation. In the latter case, particularly, they may be extremely small, so small in fact that some authors have believed them occasionally to be quite ultra-microscopic. When placed under a favorable environment they may develop into the typical bacterial cell.

Not infrequently bacterial cells which do not produce typical endospores may nevertheless be found to become relatively resistant to unfavorable conditions, such as desiccation. In the laboratory, for example, it may be readily demonstrated that certain cells of cocci may be dried, and blown about in the

atmosphere for a time, and still retain the ability to produce growth under favorable conditions. Such cells, which are morphologically little differentiated from the vegetative cells, but which are more resistant, are sometimes termed *arthrospores*.

Certain of the so-called higher or filamentous bacteria produce numbers of specialized cells or spores termed *conidia* (Fig. 20). These are formed usually at the free tip of the filament. In some cases the tip successively pinches off reproductive cells, in others a group of cells is differentiated at this point. These spores resemble very closely those produced by certain types of molds. In fact this group of trichobacteria is probably intermediate between true bacteria and molds (Fig. 21).

FIG. 21. — Spore formation by trichobacteria. *A*, Crenothrix; B_{1-4}, Actinomyces.

Spores are usually much more resistant to unfavorable conditions, such as drying, heat, light, and chemical agents, than are the cells which produced them. This is probably due to two factors: the greater protection afforded by the spore membrane and the fact that the protoplasm of the spore contains much less water than that of the vegetative cell. It is a well-known physiological fact that a moist cell is more easily destroyed than one which is dry. Freezing, for example, will seriously injure a moist wheat kernel, but is without material effect upon one which is dry.

CHAPTER IV

HOW BACTERIA ARE NAMED AND CLASSIFIED

How Plants and Animals are named. — Animals and plants (including all those forms studied in bacteriology) are named in two different ways. Each one is supposed to possess a so-called scientific name which is international. It may also possess one or more common names. These common names (sometimes called trivial, casual, or vernacular names) are usually only local. The same plant or animal usually has different common names in different languages. Manifestly, for purposes of science, it is highly desirable that the same plant or animal should possess a scientific name which would be the same in all countries. Various suggestions have been made as to how this may best be accomplished. There is in modern times, however, a general agreement that it is best to follow the leadership of a Swedish biologist, Carl von Linné (usually written in its latinized form Linnæus). This student of botany and zoölogy between the years 1750 and 1760 evolved what has come to be known as the binomial system of naming plants and animals (system of binomial nomenclature). The language of science at that time was generally Latin. In consequence, Latin was chosen as the language for the naming of plants and animals generally. Since the time of Linnæus, botanists and zoölogists and, of course, bacteriologists have more or less consistently attempted to follow this system. While it has some disadvantages, they are far outweighed by the advantages. Sets of rules and regulations or so-called codes had been drawn up by international congresses of zoölogists and of botanists to regulate the naming of plants and animals. These regulations are numerous and somewhat complicated. One who is a professional bacteriologist must needs know much about these regulations if he is to learn how to name the bacteria with which he comes in contact and particularly

to give new names to any new kinds which he may discover. For those who are not professional bacteriologists such extensive knowledge of the rules is quite unnecessary, but a few of them should be understood. Unfortunately many who have written on the subject of bacteriology have not understood and used these few simple rules, and consequently the names for bacteria used by different authorities show such differences that they are apt to prove very confusing to the beginning student.

1. The scientific name of any plant or animal is made up of two words, both in Latin. For example, the scientific name of the microörganism which produces inflammation and pus in wounds most frequently is *Staphylococcus aureus*.

2. The first of these two words is a proper noun always spelled with a capital letter and indicating the name of the group or *genus* to which it belongs. In the preceding example, the generic name is Staphylococcus.

3. The second word is the one which designates the particular kind or species. It is usually an adjective, or a noun in the genitive case modifying the generic name, or occasionally a noun in apposition with the generic name. This specific name is never capitalized unless it is derived from a proper noun, and capitalization is then optional. If the specific name is an adjective, it must agree with the generic name in gender in accordance with the rules of Latin.

Particular attention should be called to the statement made above that the scientific name is not supposed to consist of more than two words, that is, trinomials and polynomials should be avoided. Occasionally a trinomial is necessary when it takes two words to express a single idea; for example, one organism which produces lactic acid was named *Bacterium acidi lactici*. In this case the two words together express a single concept. Unfortunately not all bacteriologists have adhered to this simple rule, and in consequence some very awkward and complex designations are to be found in the literature of bacteriology. It should be understood that examples such as the following are invalid and are a clear indication of the lack of adherence to well-recognized rules on the part of those using them. Names, for example, such as the following have been used: *Bacillus membranaceus amethy-*

stinus mobilis and *Bacillus saccharobutyricus fluorescens liquefaciens immobilis*. Such names controvert plainly fundamental principles of biological nomenclature. Fortunately yeasts and molds have rarely been designated by such cumbersome titles.

Frequently pseudo-scientific names have been given to bacteria of considerable economic importance. The organism that causes pneumonia, for example, is often called the pneumococcus; that which causes cerebrospinal meningitis, the meningococcus. These names are not to be confused with scientific names. They are common names only.

Groups of Bacteria. — As was noted above, every kind of an organism is called a species, and every individual therefore is supposed to belong to a species. Related species are grouped together into genera. For example, the microörganism which causes tuberculosis seems to be rather closely related to the species which causes leprosy. They are therefore put together in a genus called Mycobacterium. The first is called *Mycobacterium tuberculosis*, and the second, *Mycobacterium lepræ*. Similarly, it is possible to group together genera which are closely related into larger groups called families. For example, it is customary to group together the various genera of spherical bacteria such as Streptococcus, Staphylococcus, Sarcina, etc., into the family named Coccaceæ. The family name is usually made by changing the ending of the name of one of the genera belonging to it to *-aceæ*. For example, the family to which the genus Bacterium belongs is usually known as Bacteriaceæ.

Similarly related families are grouped together into orders. It is customary to name orders of plants by changing the ending of one of the constituent families to *-ales*. For example, one of the orders of bacteria is named Spirochætales from the name of one of its families, Spirochætaceæ. As will be noted below, there are generally six orders of bacteria recognized. These are in turn united into a larger group called a class. In this case the entire group of bacteria constitutes such a class and has been given the name Schizomycetes (from the Greek word meaning fission fungi).

Differentiation of Bacteria. — There are thousands of species of bacteria which have been named. In all probability a very

small proportion of all the species which exist have been discovered or described. The question at once arises how is it possible to separate and identify thousands of kinds of plants which are so small and so undifferentiated as the bacteria? Among the higher plants and animals it is customary to use morphology almost exclusively for their differentiation. One species of oak tree may be distinguished from another, for example, by differences in the shape of the tree, the shape of the leaves, the shape, color, size, and general appearance of the acorn and its cup, etc. With bacteria it is quite evident that the problem is somewhat more difficult. Practically all bacteria are comparatively simple spheres, rods, or spirals. It is quite apparent that something besides morphology must be used for their differentiation. In general it may be satisfactory, therefore, to say that kinds of bacteria are differentiated quite as frequently on the basis of *what they do* as on the basis of *what they look like*. In other words, the physiology of bacteria is quite as important as the morphology in their differentiation. Morphology is used as far as practicable: shape, size, number, and arrangement of flagella; presence or absence, and position of spores; staining characteristics; presence or absence of granules. These and many other morphological characters are used wherever possible. In some cases, however, no morphological differences can be found between two bacteria which are quite certainly distinct. For example, it is quite difficult if not impossible to discover any morphological difference between the organism which causes typhoid fever and certain food-poisoning bacteria. They can be differentiated, however, quite readily in the laboratory by the way in which they grow upon culture media or the changes which they bring about in the reaction of a medium containing sugars; their ability to produce gas and acid, etc., are all utilized in classification.

A large proportion of the bacteriological technique which is studied in the laboratory is primarily for the purpose of learning the various methods which may be used for the differentiation of the bacteria.

Classification of the Bacteria. — The student in beginning bacteriology who reads various scientific articles and various text-books on this subject will usually be troubled to find that

bacteriologists have not as yet entirely agreed upon systems of classification, and in consequence on the names to be applied to particular bacteria. He should not be surprised to find the same organism called by different names by different authorities. The system of classification that will be followed in this chapter and later in the book will be a modification of one which was suggested by a committee on nomenclature appointed by the Association of American Bacteriologists.

In the following paragraphs an outline of the classification of bacteria is presented. Keys will be given which will enable one to differentiate the various orders, families, and genera of the greatest economic significance. It should be recognized by the student that there are many genera and families which are not noted at all. Even after the elimination of a large proportion of the names, he will be puzzled to know what to do with the considerable number remaining. It is suggested that the remainder of this chapter be read with some degree of care, but that no attempt be made, initially at least, to commit it to memory. It should be regarded rather as a chapter to which the student may turn later when puzzled by the names applied to organisms. For example, one chapter in the book is devoted to the subject of the transformation of alcohol into acetic acid, that is, to vinegar manufacture, by bacteria. The organisms which bring about this change belong to the genus Acetobacter. When this chapter is studied it is wise to review the relationships of Acetobacter to the other bacteria by noting just where it belongs in the classification of the bacteria.

Short Characterizations of the Class Schizomycetes (Bacteria). — As was noted above, the class to which the bacteria belong is named Schizomycetes. The bacteria are all among the one-celled, chlorophyll-free plants which typically multiply by a process of fission. The cells may be spherical, cylindrical, comma-shaped, spiral, or filamentous, and are often united into chains or into flat or cubical aggregates.

The Orders of Bacteria. — The bacteria or Schizomycetes are usually divided into six orders. In one of these orders are placed the common or typical bacteria. In the other five orders are placed those organisms which seem to be intermediate in certain char-

acteristics between the true bacteria and other plants or animals. The name *Eubacteriales* is usually applied to the true bacteria, that is, it includes the forms which are apparently most primitive in their characteristics and are least differentiated. The second order is one in which the bacteria are more or less mold-like. It constitutes a group intermediate in many characters between the true bacteria and the molds. It is named *Actinomycetales* or mold bacteria. The third order is one which is differentiated by the possession of sheaths about the cells and by the fact that iron in some form is usually deposited in considerable amounts in such sheaths. This order is named the *Chlamydobacteriales*, or the sheath bacteria. They seem to be intermediate in certain respects

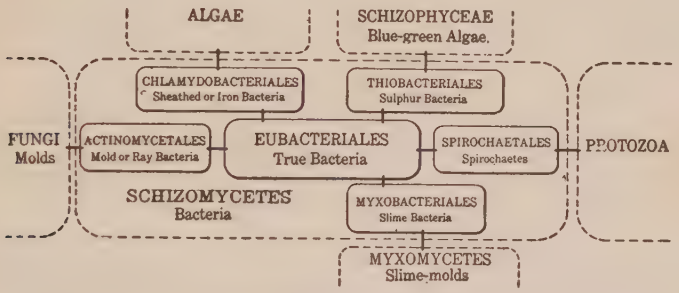


FIG. 22. — The orders of bacteria, illustrating their relationships to each other and to other groups of plants and animals.

between the true bacteria and another group of plants termed the algæ. The fourth order contains those bacteria which show the capacity for growth in water containing more or less hydrogen sulphide. Many of them contain a purple coloring material. This mode of life has given them the name *Thiobacteriales*, or sulphur bacteria. These bacteria are intermediate in many of their characters between the true bacteria and the blue-green algæ. The fifth order is *Myxobacteriales*, or slime bacteria, and constitutes a peculiar group having certain resemblances to the slime molds. It is possible that they should be listed as intermediate forms, though this is rather questionable. The last order is named *Spirochætales*, or spirochetes. It includes more or less slender, usually spiral, microörganisms which show certain char-

acteristics intermediate between the true bacteria and certain of the protozoa.

The relationships among these orders may be illustrated by Fig. 22, which is an attempt to show the relative positions of the true bacteria, the protozoa, the algæ, the blue-green algæ, the molds, and the orders of bacteria intermediate between these and the true bacteria. (See Fig. 3.) The following key to the orders may be useful in fixing their relationships. Three only among the six orders contain organisms that are of special significance in economic bacteriology. They are indicated in the key by the use of **bold faced** type. These orders are the only ones which will be discussed further. It should be recognized that those orders which are not here discussed are in some cases very large, containing many families and genera.

THE ORDERS OF BACTERIA

a. Simple and undifferentiated forms, the true bacteria . . . . . 1. **Eubacteriales**

aa. Specialized or differentiated groups

b. Plant-like

c. Mold-like . . . . . 2. **Actinomycetales**

cc. Not mold-like

d. Sheathed . . . . . 3. *Chlamydobacteriales*

dd. Not sheathed

e. Sulphur bacteria . . . . . 4. *Thiobacteriales*

ee. Slime-mold-like . . . . 5. *Myxobacteriales*

bb. Protozoan-like . . . . . 6. **Spirochætales**

THE ORDER EUBACTERIALES (THE TRUE BACTERIA)

This order was above defined as including the least differentiated and specialized of the bacteria. Most of the bacteria of economic importance belong in this order. These are the typical bacteria, with comparatively simple spherical, rod-shaped, or spiral cells. They do not show any close resemblances to other groups of plants as the molds, the algæ, or the protozoa.

It is within this order that there exists the greatest confusion

in the classification of bacteria. Usually, however, five families can be clearly differentiated. They may be recognized by the following key:

Key to the Families of the Order Eubacteriales (True Bacteria)

a. Bacteria which may be grown in the organic culture media. Not oxidizing nitrites nor ammonia
  b. Cells spherical . . . . . I. **Coccaceæ**
  bb. Cells elongate, not spherical
    c. Cells straight rods. Not bent or spiral
      d. Endospores produced . . . . II. **Bacillaceæ**
      dd. Endospores not produced . . III. **Bacteriaceæ**
    cc. Cells bent or spiral rods . . . . . IV. **Spirillaceæ**
aa. Bacteria which do not grow in the usual laboratory media containing organic matter. Ammonia or nitrites oxidized to secure growth energy . . . . . V. **Nitrobacteriaceæ**

I. The Family Coccaceae (The Cocci)

This family includes most of the bacteria whose cells are usually spherical; in some cases the cells may be somewhat elongated just preceding cell division. If the cells remain in contact after division they are frequently flattened in the plane of division. In some cases they cohere to form chains, packets, or irregular masses.

A very few species only of cocci are known to be motile. None of them are of economic importance. Endospores are never produced.

The cocci are of importance for two reasons. Some of them are active in producing acid fermentation of sugars, as in the souring of milk. Others are significant because they are parasitic and produce disease in man or animals. The six most important genera of this family may be differentiated by the following key. Two additional genera are sometimes recognized, *Leuconostoc* for cocci which produce large gelatinous masses in sugar solutions; *Rhodococcus* for cocci which are red in color, *i.e.*, produce a red pigment, and *Gaffkya* for certain parasitic cocci which generally occur in fours (tetrads).

Key to the Genera of the Family Coccaceæ (Cocci)

a. Cells occurring typically in pairs
  b. Cells are gram positive.[1] Usually capsulated . . . . . I. **Diplococcus**
  bb. Cells are gram negative.[1] Usually not capsulated . . . . . II. **Neisseria**
aa. Cells not occurring typically in pairs
  b. Cells typically in chains . . . . III. **Streptococcus**
  bb. Cells not typically in chains
    c. Cells single, or irregularly grouped
      d. Typically gram positive . . IV. **Staphylococcus**
      dd. Typically gram negative . . . V. **Micrococcus**
    cc. Cells occurring in groups of fours and eights, in cubes or regular packets . . . . . VI. **Sarcina**

Diplococcus. — The organisms belonging to this genus are strict parasites, gram positive, occurring in pairs, the cells usually somewhat pointed and encapsulated. The most important species is the organism usually associated with penumonia, the *Diplococcus pneumoniæ.*

Neisseria. — The organisms of the genus are strict parasites, growing rather poorly upon artificial culture media. The cells commonly occur in pairs, flattened on the proximal side, usually coffee-bean shaped, and gram negative. Two of the diseases produced by organisms of this group are of importance, namely, cerebro-spinal meningitis and gonorrhea, caused by *Neisseria meningitidis* and *N. gonorrhœæ* respectively.

Streptococcus. — This genus includes those spherical bacteria whose cells occur normally in chains. Certain of the species are important in that they bring about lactic acid fermentation in milk (as *Streptococcus lactis*); others are capable of producing disease (as *Streptococcus pyogenes*) in certain wound infections (Fig. 23).

Sarcina. — Organisms belonging to this genus have their spherical cells usually massed in regular packets, consisting of cubes of eight cells each (Fig. 24). When grown in the laboratory, pig-

[1] The terms gram positive and gram negative refer to a special staining method applied to cells and the results secured. This is discussed on page 154.

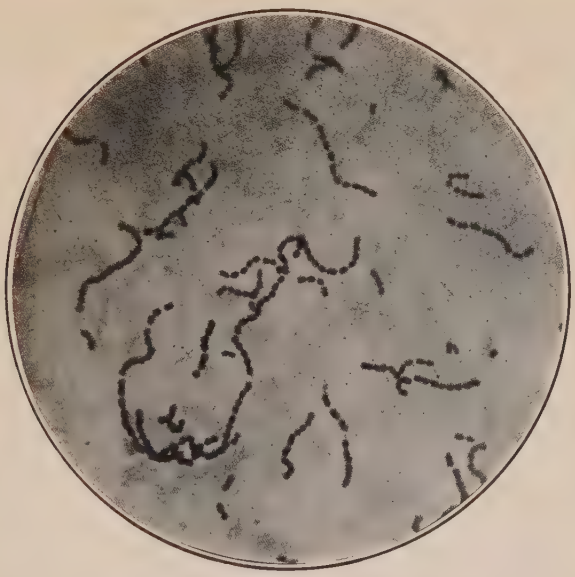


FIG. 23. — A Streptococcus from milk. (Photomicrograph, × 1000.)

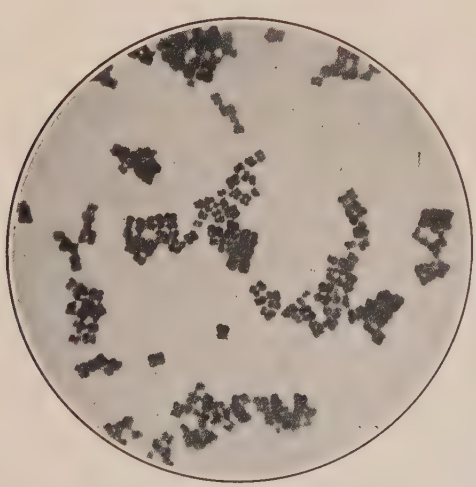


FIG. 24. — Sarcina, from colony on plate exposed to the air. (Photomicrograph, × 1000.)

ment is frequently developed, usually yellow in color. None are disease producers. One common species is *Sarcina lutea.*

Staphylococcus. — In this genus the spherical cells are united into more or less irregular masses. They are gram positive, and usually parasitic. They are commonly associated with pus production, wound infection, the development of boils, abscesses, and similar conditions in the body of man and animals. The most important species is *Staphylococcus aureus* (Fig. 25).

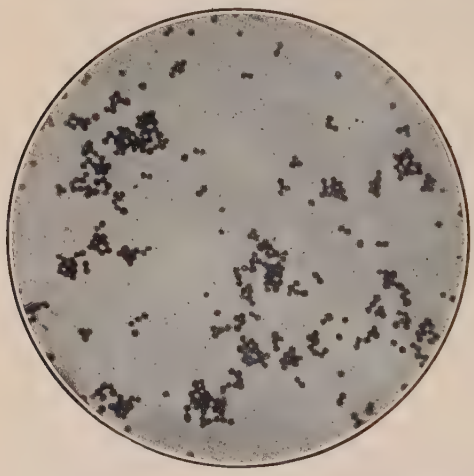


FIG. 25. — Staphylococcus. (Photomicrograph, × 1000.)

Micrococcus. — The organisms of this genus are usually not parasitic, but are common in water and soil. The cells are irregularly grouped. They are usually gram negative, occasionally motile, and when grown in the laboratory often develop a yellow pigment. There are no species of considerable economic importance.

II. The Family Bacillaceæ

The organisms belonging to this family are all rod-shaped and all produce endospores. Some forms are motile, others are nonmotile. When motile, the flagella are peritrichous. The cells may occur singly or they may be in chains. Some species are capsulated.

Bacteriologists are not in entire agreement as to the number of genera which should be recognized in this family. Some recognize only a single genus Bacillus, including in it all spore-producing, rod-shaped bacteria. Others differentiate two genera; Bacillus for those which will grow in contact with air (oxygen), *i.e.*, are *aërobic*, and Clostridium for those that grow only or best in the

absence of oxygen, *i.e.*, are *anaërobic*. Sometimes other genera are recognized, as Aërobacillus for those aërobic rods which will grow also in the absence of air, and ferment carbohydrates with production of gas. Other genera of anaërobes are sometimes included.

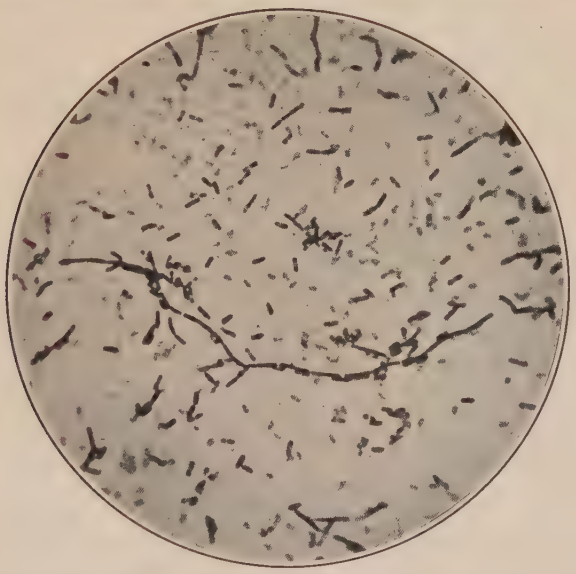


FIG. 26. — *Bacillus subtilis*. — Note the long chain of rods, and the numerous spores. (× 1000.)

Most commonly the two genera as differentiated in the following key are used.

Key to the Genera of the Family Bacillaceæ

a. Aërobic or facultative rods, *i.e.*, capable of growing in contact with air, some forms also grow in absence of free oxygen . . . . . I. **Bacillus**

b. Anaërobic rods, not growing in contact with free oxygen . . . . . II. **Clostridium**

Bacillus. — The organisms belonging to this genus are all rod shaped, sometimes occurring in chains. The spores are produced readily (Fig. 26). These spores are usually relatively resistant

to unfavorable conditions. All species are aërobic; some are also facultative. Under the right conditions these bacteria are among the most active in nature in producing decomposition of organic materials. Some produce fermentation of carbohydrates; these are sometimes separated as the genus Aërobacillus. One species (*Bacillus anthracis*) produces the disease anthrax in animals and man.

Clostridium. — The organisms of this genus are all rod shaped, and produce endospores but do not grow in the presence of free atmospheric oxygen. Frequently the rods are swollen at time of spore production, forming spindle-shaped or club-shaped cells. Some of the bacteria of this group are among the most active of the putrefactive forms, particularly in the development of malodorous compounds such as butyric acid (as by *Clostridium butyricum*). Others are important in certain fermentations, as production of butyl alcohol. Other species are capable of producing disease, particularly when introduced into wounds. Among the diseases produced are gaseous gangrene in man, and malignant edema and blackleg in animals.

III. The Family Bacteriaceæ

The organisms of this family are rod shaped, but do not produce endospores. Some are motile, some non-motile.

This family is the largest in number of genera and of species among the families of the true bacteria. It is likewise the family in which there is the greatest diversity in nomenclature. The classification of genera here given is as short and simple as is practicable, but there are several principal reasons why any classification outlined may prove unsatisfactory. Bacteriologists are not agreed as to the number of genera which should be recognized. Some desire to hold the bacteria together in genera containing a large number of species, others would split the large genera into two or many smaller genera. It is thought best here to differentiate in a key between the large genera, and in the subsequent description of the genera to indicate the manner in which in each case the genus may be split into smaller genera.

From an economic point of view the bacteria of this family are the most important. Some of them are useful in the soil, in the preparation of foods, in producing desirable fermentations; in other cases they cause putrefactions, undesirable fermentations, and diseases in man, animals, and plants.

Key to the Genera of the Family Bacteriaceæ [1]

a. Bacteria which are able aërobically to utilize (fix) atmospheric nitrogen
  b. Usually found growing in the nodules on the roots of leguminous plants . . . . . I. **Rhizobium**
  bb. Living free in the soil, non-symbiotic . II. **Azotobacter**
aa. Bacteria which do not fix appreciable amounts of atmospheric nitrogen
  b. Bacteria which actively oxidize ethyl alcohol to acetic acid . . . . . III. **Acetobacter**
  bb. Bacteria which do not change alcohol to acetic acid
    c. Bacteria usually motile by means of polar flagella, often with a fluorescent pigment . . . IV. **Pseudomonas**
    cc. Flagella, if present, not polar
      d. Cells brightly colored (pigment-producers)
        e. Color red . . . . . V. **Serratia**
        ee. Color violet . . . VI. **Chromobacterium**
        eee. Color yellow . . . . VII. **Flavobacterium**
      dd. Cells not brightly colored
        e. Cells gram positive. Attacking carbohydrates, with acid (usually lactic acid) production . . . . . VIII. **Lactobacillus** [2]
        ee. Cells gram negative
          f. Requiring serum or hemoglobin for growth . . . . . IX. **Hemophilus**
          ff. Not requiring serum or hemoglobin

[1] Other genera not here listed are: — *Thiobacillus*, which includes bacteria oxidizing free sulphur or sulphur compounds, *Hydrogenomonas* which oxidizes gaseous hydrogen, *Methanomonas* which oxidizes methane, and *Carboxydomonas* which oxidizes carbon monoxide. The validity of the last three named is somewhat problematical.

[2] The generic name *Kurthia* (sometimes *Zopfius*) is used to designate gram positive forms that do not attack carbohydrates.

g. Cells showing marked polar staining
. . . . . X. **Pasteurella**
gg. Cells not showing marked polar staining
. . . . . XI. **Bacterium**

Rhizobium. — The organisms belonging to this genus are minute rods, motile when young by means of flagella which may be either polar or peritrichous. The cells may occur free in the soil, but usually are present in the characteristic nodules on the roots of leguminous plants. By use of the energy secured by the oxidation of carbon compounds, usually sugars, they are capable of fixing atmospheric nitrogen. The species found generally upon the roots of leguminous plants is *Rhizobium leguminosarum*. It is frequently mentioned in literature as the *Bacillus radicicola*. The organism has been claimed to be able to fix atmospheric nitrogen when grown in the laboratory in solutions containing carbohydrates.

Azotobacter. — Organisms belonging to this genus are relatively large rods, sometimes almost spherical, and occasionally yeast-like in appearance. Some species (as *Azotobacter chroococcum*) are typically soil forms, securing their growth energy by the oxidation of carbonaceous material, and are of unusual importance because of their ability to fix atmospheric nitrogen for their own use, thus increasing the nitrogen content of soils. They do not live upon the roots of leguminous plants, but are free in the soil. When grown in the laboratory their ability to use atmospheric nitrogen may be shown by cultivating them in solutions containing carbohydrates but deficient in nitrogen compounds.

Acetobacter. — The organisms are rod shaped, frequently in chains, and either motile or non-motile. The bacteria grow on the surface of alcoholic solutions, securing the energy for their growth usually by the oxidation of alcohol to acetic acid, or occasionally by the oxidation of sugars to acids. Involution forms are not infrequent, occurring as filamentous, club-shaped, and even branched cells. Organisms belonging to this genus (such as *Acetobacter aceti*) constitute the so-called *mother of vinegar* which forms over the surface of cider or wine in the process of vinegar manufacture.

Pseudomonas. — The cells are rod shaped, and usually motile by means of polar flagella. Frequently a fluorescent green or brown pigment is produced in culture media. In other species an insoluble yellow pigment is formed. Among those producing a green fluorescent pigment is the *Pseudomonas æruginosa*, an organism sometimes found in wounds. Many of the yellow species produce disease in plants. These are sometimes separated as a genus *Phytomonas*.

Serratia. — This genus is closely related to the genus Bacterium discussed below; it differs primarily in the possession of a brilliant red pigment. The first species described was one which produced red or "bloody" spots in bread; it was named *Serratia marcescens* in 1823. This genus is also sometimes known as *Erythrobacillus*.

Chromobacterium. — This genus includes certain species of rod-shaped bacteria frequently isolated from water. The species *Chromobacterium violaceum* is not uncommon in this habitat.

Flavobacterium. — The species of this genus are differentiated primarily from those of the genus Bacterium by the possession of a yellow to orange pigment. Most of the species (such as *Flavobacterium aquatilis*) are described from water or soil.

Lactobacillus. — The cells are rod shaped, often long and relatively slender, gram positive, and non-motile. Some species have very short cells and are almost coccus-like. No endospores are developed. Usually acid, particularly lactic acid, is produced in considerable quantities from carbohydrates. Many of the species grow best in the absence of oxygen although they are not strictly anaërobic. Included in this genus are many species of bacteria important in the souring of milk, the formation of lactic acid in silage, sauerkraut, etc.

Among the important species are *Lactobacillus bulgaricus* and *L. casei*. A genus, *Propionobacterium* is sometimes recognized to include the closely related bacteria which produce propionic acid instead of lactic acid.

Hemophilus. — The organisms belonging to this genus are minute, parasitic, gram negative rods, which grow in the laboratory only in special media, preferably containing hemoglobin.

The most important species is *Hemophilus influenzæ*, the organism which has been supposed to cause influenza and which is quite certainly associated at least with many cases of the disease.

Pasteurella. — The organisms of this genus are all non-motile rods, gram negative and showing bipolar staining. They are parasitic with very slight powers of fermentation. Members of this genus produce many diseases in animals, such as fowl cholera, and related diseases in swine, sheep, cattle, and horses, and one (*Pasteurella pestis*) the disease, bubonic plague, in man.

Bacterium. — The organisms belonging to this genus are gram negative rods, frequently motile, and easily cultivable. Most species ferment certain carbohydrates with the formation of acid and frequently gas. They are typically intestinal parasites in man and higher animals. Some species are not infrequent in the soil. A few species are pathogenic, including the organisms causing typhoid fever, para-typhoid fever, dysentery, and certain types of food poisoning. One species, the *Bacterium coli*, is frequently used as an index for the detection of sewage in water.

This genus as defined above is a very large one, so large, and with so numerous and diverse species that there is an increasing tendency among bacteriologists to divide it up into a considerable number of genera. Those which are most frequently recognized are differentiated in the following key. It will be noted that ability to ferment sugars with gas and acid production are important criteria in the differentiation.

Key to the Genera Most Commonly Recognized When the Genus Bacterium Is Divided

a. Bacteria which produce acid and gas from lactose
  b. Producing acetoin (acetyl-methyl-carbinol) from dextrose . . . . . I. **Aerobacter**
  bb. Not producing acetoin from dextrose . . . . . II. **Escherichia** (in restricted sense)
aa. Bacteria which do not produce gas from lactose
  b. Producing acid and gas from sucrose . . . . . III. **Proteus**

bb. Producing no gas from sucrose

c. Producing acid and gas from dextrose
. . . . . IV. **Salmonella**

cc. Producing acid but no gas from dextrose
. . . . . V. **Eberthella**

ccc. Producing neither acid nor gas from dextrose
. . . . . VI. **Alcaligenes**

The genus *Aërobacter* includes bacteria such as *Aërobacter ærogenes*, common in soil and water. Many species are very active in the production of various fermentations. Another genus **Klebsiella** (sometimes termed **Encapsulatus**) is frequently separated from Aërobacter to include those forms showing distinct capsule formation.

One of the most important species of the genus Escherichia is *Escherichia coli* and its close relatives. These are found commonly in the intestines of man and animals, and are in consequence present in abundance in sewage. They will be discussed in detail later in connection with the detection of sewage pollution in water.

The commonest species of Proteus is probably *Proteus vulgaris*, an organism frequently found associated with decay. Most species are able to liquefy gelatin rapidly.

The genus Salmonella contains a number of disease-producing bacteria such as *Salmonella enteritidis*, the organism frequently responsible for food poisoning.

Eberthella includes largely disease-producing bacteria, such as those causing typhoid fever (*Eberthella typhosa*) and dysentery. The latter, largely on the basis of their lack of motility, are sometimes separated into another genus, **Shigella.**

The genus Alcaligenes was created to contain those forms which showed very slight powers, if any, of carbohydrate fermentation. The first species described was *Alcaligenes fecalis*. Later the organisms responsible for indulant (Malta) fever in man and contagious abortion in cattle were found to belong here. The latter are now frequently separated with the generic name **Brucella.**

In addition to the genera above mentioned, several others are sometimes used. Among these are: —**Erwinia** to include those

forms belonging in this general group which are capable of producing plant diseases, **Cellulomonas** to include those species which show high powers of dissolving cellulose, and **Achromobacter** for those which are colorless, and seem to fit nowhere else. The last named genus may be regarded as a sort of a catch-all, and not very well characterized.

IV. The Family Spirillaceæ

The bacteria of this family are all rods which are more or less curved, the longer forms developing into spirals. They are motile by means of polar flagella. They do not form spores. The two genera may be separated by means of the following key.

Key to the Genera of the Family Spirillaceæ

a. Relatively short, curved rods, motile by means of one or few polar flagella . . . . . I. **Vibrio**

aa. Relatively longer, spiral rods, motile by means of a cluster of polar usually numerous flagella . . . . . II. **Spirillum**

Vibrio. — This genus includes short curved rods, motile by means of one, two, or three polar flagella. Many are intestinal parasites and some are capable of causing disease. The most important organism is the *Vibrio choleræ* producing Asiatic cholera in man. This genus is closely related to the genus Pseudomonas, resembling it in its possession of one or few polar flagella. It differs principally in that the cells are usually somewhat curved.

Spirillum. — Organisms belonging to this group are longer spiral cells, with a tuft of relatively long flagella at the tip. They are not uncommon in water, particularly in water containing considerable amounts of organic matter. None of the species is of much economic importance. Some of the species are apparently closely related to some of the spiral sulphur bacteria. The genus Spirillum may be regarded as a connecting link to the Thiobacteriales.

V. The Family Nitrobacteriaceæ

This family contains two genera of considerable significance from the standpoint of soil bacteriology. The organisms belong-

ing to this family are not cultivable in the usual laboratory media. They secure growth energy by the oxidation of inorganic nitrogen, as ammonia or nitrites, and are autotrophic (capable of assimilating CO_2 from the air).

Key to the Genera of the Family Nitrobacteriaceæ

a. Bacteria which secure growth energy by the oxidation of ammonia to nitrites . . . . . **I. Nitrosomonas**

aa. Bacteria which oxidize nitrites to nitrates . . . . . **II. Nitrobacter**

Nitrosomonas. — The bacteria of this genus oxidize ammonia to nitrites. They are important in soils and in sewage disposal. The type species is *Nitrosomonas europaea*. Some species are rod shaped, and motile by means of polar flagella, others are cocci and non-motile. The latter are sometimes differentiated as a distinct genus, *Nitrosococcus*.

Nitrobacter. — A single species of this genus (*Nitrobacter winogradskyi*) is important in soil and water in transforming nitrites into nitrates. The cells are rod shaped and non-motile.

THE ORDER ACTINOMYCETALES

The organisms of the order *Actinomycetales* usually have the cells somewhat elongated, frequently filamentous and with a decided tendency to the formation of branches. In some genera a definitely branched mycelium is developed. The cells frequently show swelling, or are clubbed or irregular in shape. Endospores are not produced, although conidia are formed by some genera. Many of the genera are gram positive and all are non-motile. Some species are parasitic in animals or in plants.

Two families of this order may be differentiated by the following key.

Key to the Families of the Order Actinomycetales

a. Filamentous and mold-like. Often branched. Conidia present in some genera . . . . . **I. Actinomycetaceæ**

aa. Rod shaped, rarely forming long filaments, rarely branched, no conidia produced . . . . . **II. Mycobacteriaceæ**

The Family Actinomycetaceæ

This is the family of bacteria which shows the greatest resemblance to the molds. Four genera are usually included in this family. One only of these, *Actinomyces*, will be separately discussed. The others are: (1) *Actinobacillus*, for a rod-filamentous type of organism, the chief species, *Actinobacillus lignieresi*, producing in cattle a disease termed actinobacillosis, (2) *Leptotrichia*, for certain filamentous bacteria (as *Leptotrichia buccalis*) found in the mouth, and (3) *Erysipelothrix*, for some filamentous forms producing certain diseases, as swine erysipelas and erysipeloid in man.

Actinomyces. — The organisms belonging to this genus produce a typical mold-like, much-branched mass of threads, and frequently develop aërial conidia as illustrated in Fig. 21. They are abundant in soils. A few species are known to produce disease; the *Actinomyces bovis* is associated with lumpy jaw of cattle.

The Family Mycobacteriaceæ

Most (though not all) of the bacteria belonging to this family are parasitic forms, frequently producing disease. They are non-motile, non-sporulating rods with a tendency to the formation of filaments, sometimes branched. Two genera, *Corynebacterium* and *Mycobacterium* are sufficiently important to require differentiation and discussion. Two other genera are included in this family, *Fusiformis* for the spindle-shaped bacteria sometimes occurring in mouth infections (as the *Fusiformis dentium* which is associated with a spirochete in Vincent's angina), and *Pfeifferella* for the species *Pfeifferella mallei*, the cause of the disease glanders in equines.

Key to Two Genera of the Family Mycobacteriaceæ

a. Cells usually showing deeply staining metachromatic granules. Not acid fast . . . . . **I. Corynebacterium**

aa. Cells acid fast . . . . . **II. Mycobacterium**

Corynebacterium. — The cells of the bacteria belonging to this genus are slender, often slightly curved, rods with a tendency

toward the formation of clubs, and with granules giving the cells when stained a barred or irregular appearance. They are not acid fast but are gram positive and aërobic. Most species are parasites, the most important being *Corynebacterium diphtheriæ*, the cause of diphtheria in man.

Mycobacterium. — The organisms of the genus *Mycobacterium* are slender rods which are stained with difficulty, but when once stained are resistant to acids, that is, they are termed *acid fast.* The cells are sometimes swollen, showing club shapes, or forked forms; occasionally the cells are branched. They are non-motile and gram positive. Some of the species are present in the soil. A few species are pathogenic to man and animals. The most important of the diseases caused are tuberculosis and leprosy, the causal organism being *Mycobacterium tuberculosis* and *M. lepræ* respectively.

THE ORDER CHLAMYDOBACTERIALES (THE SHEATHED BACTERIA)

The bacteria which belong to this order are frequently designated as the iron bacteria. For the most part they are filamentous forms in which the cells are more or less completely sheathed, and in which the sheath becomes heavily coated with iron in the form of the hydroxide or carbonate. One family only, the *Chlamydobacteriaceæ* is included. This in turn includes several genera. None of them is commonly grown in the laboratory. Some species belonging to the genera *Leptothrix* and *Cladothrix* are common in water containing considerable amounts of iron.

THE ORDER THIOBACTERIALES (THE SULPHUR BACTERIA)

This order includes a great many bacteria which have a marked resemblance on the one hand to the true bacteria, and on the other to the blue-green algæ. They are of many shapes, cocci, rods, spirals, and filaments. In general they grow in situations in nature where there is a considerable amount of hydrogen sulphide, as in the water from sulphur springs. Some species show granules of

sulphur within the cell, some possess a purplish coloring matter called bacterio-purpurin, which possibly functions for them as does the chlorophyll for green plants. All forms possess either the sulphur granules, the pigment, or both.

The order is a very large one. At least three families have been differentiated, and about thirty genera recognized. For the most part, the sulphur bacteria have not been cultivated in the laboratory. While they are undoubtedly of importance in nature, nevertheless they are so little known that they are considered only incidentally in a course in elementary bacteriology. Keys to the families and genera will not therefore be included.

THE ORDER MYXOBACTERIALES (THE SLIME BACTERIA)

This is a group of bacteria which has been studied comparatively little. They differ from the other bacteria in having a surprisingly

FIG. 27. — Bacteria belonging to the order Myxobacteriales. 1, 2, 3, stages in the development of the cysts; 4, cysts of another species; 4*a*, cyst broken open to show bacteria; 5*a*, cyst germinating, with bacteria escaping; 5*c*, cells of bacteria.

complex life cycle. The individual cells resemble those of the true bacteria. They are surrounded by a gelatinous excretion, forming slimy colonies or masses. When conditions are right, this mass develops into a relatively complex fruiting body. In some cases the entire mass develops into a cyst, or group of cysts, with the bacteria in the interior. In some forms these cysts develop on the ends of stalks, giving a superficial resemblance to

the slime molds. These encysted bacteria function as spores; when the cysts break the enclosed bacteria are scattered, and develop into new colonies. The group is sometimes termed the *Polyangidæ* after the first genus, *Polyangium* described. Several other genera are recognized. The group is apparently of no great economic importance and will not be further discussed (Fig. 27).

THE ORDER SPIROCHAETALES (THE SPIROCHETES)

The organisms belonging to this group in many respects resemble the protozoa, and may be regarded as intermediate between them and the true bacteria. They are all more or less curved rods, frequently very slender. Many species are motile but there has been no definite demonstration of flagella. They multiply either by longitudinal or transverse fission. It is probable that in some cases they have a relatively complex life history. Several genera have been described, the most important being *Treponema*, *Leptospira*, and *Borrelia* (*Spironema*).

Treponema. — The organisms belonging to this genus are exceedingly slender spiral rods, motile by means of flexuous bending of the body. The most important species is *Treponema pallidum*, the cause of the disease syphilis in man.

CHAPTER V

MORPHOLOGY OF THE YEASTS

THE yeasts resemble the bacteria in that they are unicellular organisms, but differ to some degree in their cell structure, grouping, size, sporulation and multiplication. The yeast cell may be ovoid, ellipsoidal, or cylindrical, more rarely spherical or considerably elongated (filamentous). The shape, while fairly constant for a given species, nevertheless exhibits considerable variation.

The grouping of the cells shows little of the regularity to be noted among the bacteria. The yeasts usually multiply by a type of vegetative budding from any side of the cell. The daughter cells are at first small, but rapidly develop and themselves begin budding. These cells frequently cling together for a time, forming irregular masses. Sometimes the cells are elongate-cylindric and are united in chains or filaments. These closely resemble the hyphæ produced by certain molds, and constitute a type of plant structure intermediate between that of the typical yeast and the mold.

Size. — The smallest yeasts are no larger than some of the bacteria, others are very much larger. The larger size of the yeast cell is sufficiently characteristic to be one of the most useful criteria in differentiation of yeasts and bacteria. The commoner yeasts are $3 - 10\,\mu \times 3 - 100\,\mu$.

Structure. — *Cell Wall.* — The cell wall of the yeast is a more or less firm membrane of a substance frequently called yeast cellulose. The cell wall is apparently absent from the very young cell, but begins to show as a delicate membrane by the time the cell is one-third grown. The composition of the cell wall is not well understood; probably it is carbohydrate in nature although it does not give the reactions characteristic of true cellulose. The wall is often greatly thickened in old cells. Gelatinous capsules

of essentially the same character as those found among the bacteria may be present, surrounding a yeast cell. Flagella are never produced, and the yeast cells are never motile.

Cell Contents. — The protoplasm or living contents of the yeast cell may be separated into three components: the ectoplast, the cytoplasm, and the nucleus (Fig. 28). In addition certain cell inclusions are to be noted, particularly vacuoles, granules, and oil globules. The *ectoplast* in the yeasts, as in the bacteria, is the outer differentiated layer of the protoplasm lying just within the cell wall and appressed to it. It undoubtedly acts as a semi-permeable or osmotic membrane, and determines what may enter and what may leave the protoplasm of the cell. The presence of a definite *nucleus* in the yeast cell differentiates it from a bacterium. The nucleus is small relative to the size of the cell, and is recognized with difficulty, if at all, in the unstained specimen, although proper staining methods show it distinctly. The nucleus divides preceding the formation of new cells, one-half going to the daughter cell. The nuclear division is a primitive type of mitosis. The *cytoplasm* of all mature yeast cells is *vacuolate;* that is, it contains spaces filled with cell sap and not living substances. Granules of *glycogen* and globules of *oil* may also be found, the former probably representing reserve food supply, the latter stored for the same purpose or possibly the result of degenerative processes.

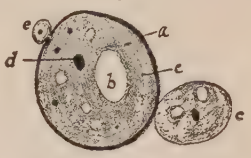


FIG. 28. — Morphology of the yeast cell. *a*, cell wall; *b*, vacuole; *c*, granules; *d*, nucleus; *e* and *e*, buds.

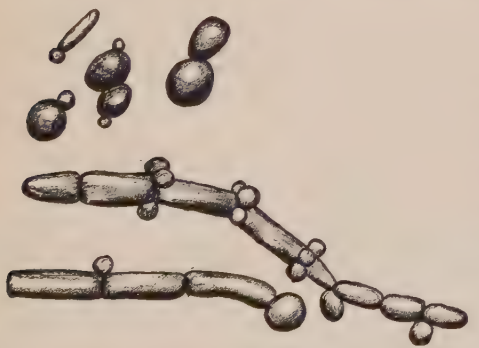


FIG. 29. — Various types of budding yeast cells. (Adapted from Hansen.)

Reproduction of Yeasts. — True yeasts reproduce both vegetatively and by means of spores.

Vegetative Reproduction. — The true yeasts reproduce vegetatively in one of two ways, by budding or by fission. It will be shown later that this difference in the method constitutes the basis for a division of these true yeasts into two groups. In the first method a minute protuberance appears on one side of the mother cell, the nucleus divides, and one portion passes into this bud. The bud then enlarges and is separated by a constriction from the mother cell. At first no cell wall is demonstrable, but this soon develops. The daughter cell may separate at once and become entirely free or it may remain attached for a time. This type of multiplication is illustrated in Fig. 29 and Fig. 30.

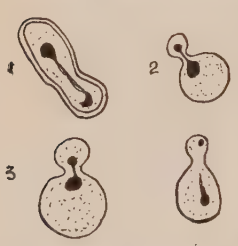


FIG. 30. — Participation of the nucleus in the budding of the yeast cell. 1, *Saccharomyces ludwigii*; 2, *Saccharomyces cerevisiæ*; 3, *Saccharomyces cerevisiæ*. (1, adapted from Janssens; 2, from Guillermond; and 3, from Swellengrebel.)

Multiplication by fission in the genus Schizosaccharomyces resembles in some degree the vegetative multiplication in the bacteria. The cell develops to its full size, and a membrane forms across the middle. A dividing cell wall is produced and the two cells separate. (See Fig. 31.)

FIG. 31. — Schizosaccharomyces, vegetative reproduction by fission.

Spore Production. — All true yeasts produce spores. As will be noted later, the yeasts are to be regarded as simple, perhaps primitive members of that order of fungi called Ascomycetes or sac fungi. This order is characterized by the formation of a number of spores within a cell, frequently one that has been somewhat differentiated for the purpose. In the yeasts, the spores develop within the cells that are little or not at all different from the vegetative cells. A cell containing spores is termed an *ascus* or sac, the spores are called *ascospores* or more rarely *endospores*. The types of asci and ascospores found in yeasts are shown in Fig. 32.

Yeasts require suitable conditions for production of spores; they are rarely to be found in actively growing cultures in nutrient solutions. E. Chr. Hansen has formulated the following general

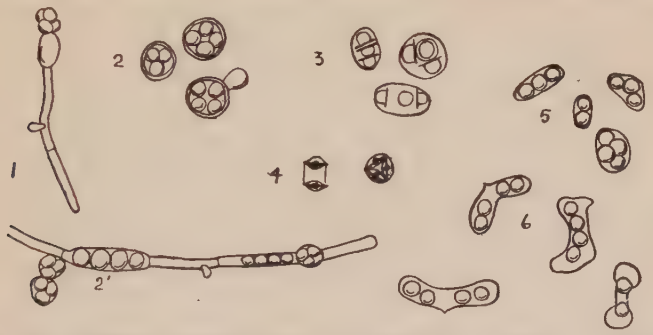


FIG. 32. — Different types of asci and ascospores in yeasts. 1, *Saccharomycopsis capsularis;* 2, *Saccharomyces cerevisiæ;* 3, *Saccharomyces anomalus;* 4, *Saccharomyces saturnus;* 5, 6, wine yeasts. (1, adapted from Schiönning; 4, from Klöcker; and 2, 3, 5, and 6 from Hansen.)

rules for inducing spore production. (1) The cells must come from a young, well-nourished culture. (2) The culture must be well aërated. (3) There must be an abundance of moisture. (4) The temperature must be somewhat high, the optimum for most types being about 25° C. These conditions are met by the use of a sterile block of plaster of paris set in sterile water with the upper surface exposed to the air. The top is seeded with yeast from a vigorous, young, well-nourished culture and placed at 25° C. for twenty-four to forty-eight hours. Usually the spores will have developed in this time. Spores may sometimes be found in films of yeasts floating on nutrient solutions or in cultures on solid media.

FIG. 33. — Participation of the cell nucleus in the formation of spores in Saccharomyces. *a*, mother cell with single nucleus; *b*, nucleus just completing first division; *c*, division of nucleus completed; *d*, mother cell with four nuclei; *e*, mother cell with eight nuclei; *f*, each nucleus has surrounded itself with protoplasm and a cell wall and constitutes a spore (an ascospore) and the mother cell wall an ascus (or sac). (Adapted from Lindau.)

Usually a constant number of spores will develop in the cells of a given species, but this is not invariable. Some yeasts develop one spore only; usually there are from two to eight, rarely more within a cell. The spores are formed by a division of the nucleus into a number of nuclei corresponding with the number of the spores to be developed. The participation of the nucleus in the formation of the ascospores is illustrated in Fig. 33. Each then surrounds itself by a portion of the cytoplasm, and this in turn by a spore membrane. Sometimes the ascus or mother cell disintegrates after the formation of the spores; usually, however, the old wall is relatively persistent. The spores may be spherical, ellipsoidal, kidney shaped, spindle shaped, or flattened on one side. They may be smooth or banded by lines or rings.

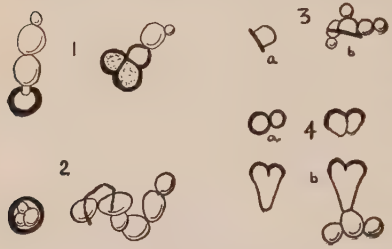


FIG. 34. — Germination of yeast spores. 1, *Saccharomyces cerevisiæ*, germination of the spores by budding; 2, same: *a*, ascus with spores; *b*, shows the old ascus or mother cell wall, with spores escaped and swollen, ready for germination; 3, *Saccharomyces anomalus: a*, single spore; *b*, spore germinated; 4, *Saccharomyces ludwigii: a*, spores; *b*, same after 30 hours. (Adapted from Hansen.)

In a few cases (*Zygosaccharomyces* and *Schizosaccharomyces*) the formation of spores is preceded by a fusion of two cells, a kind of primitive sexual act.

Ascospores germinate by swelling, or by bursting the spore wall, and budding. In some species the germinating spores fuse in pairs. This is probably a primitive type of sexual reproduction.

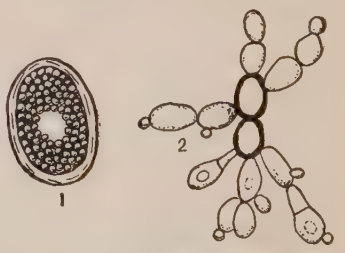


FIG. 35. — Chlamydospores or resting cells of the yeast. 1, single chamydospore with granular contents; 2, two chlamydospores germinating, giving rise to a colony of budding yeast cells. (Adapted from Will.)

In addition to the true spores, yeasts sometimes produce heavy-walled resting cells (*chlamydospores*) which function to carry the organism over periods unfavorable for growth.

CHAPTER VI

CLASSIFICATION OF THE YEASTS

True and False Yeasts. — The true yeasts are fungi that reproduce vegetatively by budding (in one genus by fission) and that form endospores (ascospores) under certain conditions. This excludes from consideration as true yeasts a large number of fungi resembling yeasts rather closely in morphology, that reproduce by budding, but do not form ascospores. Certain molds, for example, when grown in sugar solutions, produce buds and grow in much the same manner as yeasts. A few organisms morphologically resembling yeasts are known to cause disease in man and animals. Their exact botanical position is uncertain. Then there are many forms, rarely of any economic importance, that resemble true yeasts in most respects other than spore formation. These are termed pseudo-yeasts, false yeasts, torulæ, or mycodermata, and are included tentatively by the botanist among the imperfect fungi. The resemblances of certain of these forms to the true yeasts is illustrated in Fig. 36 and Fig. 37. The discussion in this chapter will be confined almost entirely to the true yeasts.

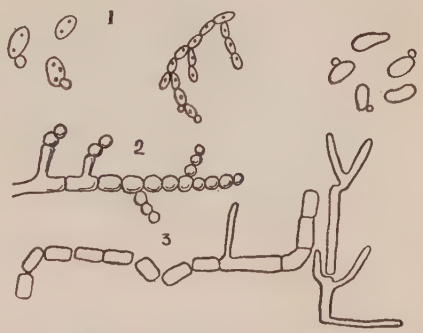


FIG. 36. — Torulæ and mycodermata. 1, Mycodermata (torulæ) from different wines; 2, budding mycelium of Mucor, showing formation of oidia; 3, *Oöspora lactis*, showing the mycelium breaking up into oidia. (1, adapted from Wisner; 2, from Wehmer; and 3, from Lindner.)

Relationship of True Yeasts to Other Microörganisms. — There is some evidence that the yeasts are rather remotely related on the one hand to the bacteria, and much more closely on the other to certain of the higher fungi. The genus Schizosaccharomyces, with its vegetative multiplication by fission and its production of endospores, has been believed by some investigators to be intermediate between the bacteria and the budding yeasts. One genus of yeast, Monospora, produces only one spore in a cell, resembling in this respect the bacterial genus Bacillus. The common method of spore production, that of ascospore formation, puts the yeasts into the class of fungi known as Ascomycetes. The yeasts differ from the other members of the class in that the cells rarely remain united in threads or hyphæ as do those of the higher forms, and in that each one of the yeast cells is potentially a spore mother cell or ascus. In the higher forms certain differentiated cells only of the fungous body become asci and form spores. The yeasts, then, are the simplest and most primitive in structure of the sac fungi.

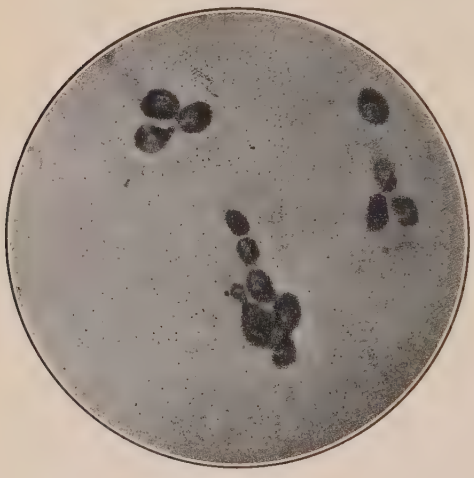


FIG. 37. — Torulæ from pickle brine. (× 1000.)

Classification of True Yeasts. — A classification of the true yeasts frequently used is that of E. Chr. Hansen in which two families and nine genera are recognized. Two, at least, of these are of doubtful value and are of no economic importance; they are therefore not included in this discussion. In the following key the genera of most importance are indicated in **boldface** type; those of minor importance in *italics*.

Key to Genera of True Yeasts

Family I. **Saccharomycetaceæ.** Vegetative reproduction by budding

A. Cells do not form a surface membrane at once on sugar media, *i.e.* do not grow exclusively at the top of the medium

1. Spores having a single membrane
   a. Cells fusing in pairs before spore formation . . . . . *Zygosaccharomyces*
   b. Cells not fusing in pairs before spore formation
      (1) Spores germinate by ordinary budding . . . . . **Saccharomyces**
      (2) Spores germinate by means of a promycelium . . . . . *Saccharomycodes*
2. Spores having two membranes . . . *Saccharomycopsis*

B. Cells forming a surface membrane at once on sugar media
1. Spores, spherical, hemispherical or irregular . *Pichia*
2. Spores lemon shaped, with pointed ends . . . *Willia*

Family II. **Schizosaccharomycetaceæ.** Vegetative reproduction by fission. One genus only . **Schizosaccharomyces**

Discussion of Genera

Zygosaccharomyces. — This genus is differentiated by the fact that the cells fuse in pairs before spore formation, representing a primitive type of sexual reproduction. Spores form readily not only on gypsum blocks, but on solid media. The two species described are of little economic importance. One was obtained from a cane sugar solution inoculated with ginger, the other from the body of the honeybee.

Fig. 38. — Zygosaccharomyces, two cells in process of conjugation showing the development of spores. (Adapted from Lafar.)

Saccharomyces. — This genus includes practically all the yeasts of economic importance. By many authors the various other genera here described are all included in this single genus Sac-

charomyces. Almost all of the species are active in the fermentation of sugar with the formation of alcohol and carbon dioxide.

There is by no means an agreement as to the best methods of differentiation of species among these yeasts. One of the first

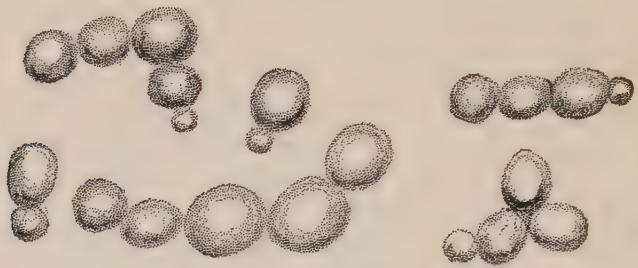


FIG. 39. — Yeast of the type of *Saccharomyces cerevisiæ*. (Adapted from Hansen.)

efforts of classification was based upon the shape of the cells. To the common bread yeast having large spherical or ovoid cells the name *Saccharomyces cerevisiæ* was given (Fig. 39); to the wine and cider yeasts having ellipsoidal or short oval cells the name *Sacch. ellipsoideus* (Fig. 40), and to all long or cylindric-celled yeasts

FIG. 40. — Yeast of the type of *Saccharomyces ellipsoideus*. (Adapted from Hansen.)

Sacch. pastorianus (Fig. 41). The extreme variability in morphology of the same yeast under different cultural conditions renders an application of such a classification difficult. These names, however, are very commonly used to express the general

morphologic type under consideration. Another classification used extensively in the brewing industry is to designate them as " bottom " or " top " yeasts. In some types of yeasts, the cells remain almost wholly below the surface of the fermenting liquid during the process of fermentation, forming a sediment on the bottom; hence the name bottom yeast. In others, the organisms form a frothy scum on the surface during the process of active fermentation, and only after fermentation has largely ceased does this superficial mass break to pieces and settle to the bottom. To this type the name top yeast is applied. Hansen has shown, however, that these characters are not constant, and concludes that this is not a satisfactory basis for classification. Physiologic characters, such as ability to ferment various sugars, and cultural characters on solid media have in recent years been given greater prominence in the formulation of classifications.

FIG. 41. — Budding yeast cells of *Saccharomyces pastorianus*.

The species of the genus Saccharomyces are often separated into six subgroups differentiated from each other on the basis of their ability or lack of ability to ferment various sugars with production of alcohol and gas as outlined below.

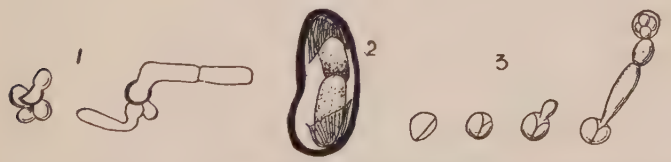


FIG. 42. — The yeast genera Saccharomycodes and Saccharomycopsis. 1, *Saccharomycodes ludwigii*, showing germination of spore by means of a promycelium; 2, *Saccharomycopsis guttulatus*, showing two spores in the ascus or mother cell, each with two membranes; 3, *Saccharomycopsis capsularis*, germination of a spore and formation of an ascus and spores. (1, adapted from Hansen; 2, from Wilhelmi; 3, from Schiönning.)

Group I ferments dextrose, sucrose, and maltose, but not lactose.

Group II ferments dextrose and sucrose, but not maltose and lactose.

Group III ferments dextrose and maltose but not sucrose and lactose.

Group IV ferments dextrose but not maltose, sucrose and lactose.

Group V ferments dextrose and lactose.

Group VI ferments none of these four sugars.

Most of the yeasts of economic importance in the fermentation industries, such as bread making, brewing, wine making, and commercial manufacture of alcohol are found in Group I. Many of these have never received true specific names, but are commonly designated by the name of the locality from which they come, as Carlsberg bottom yeast No. 2 and Johannisberg II. Group V includes certain yeasts that have been found active in the alcoholic fermentation of milk.

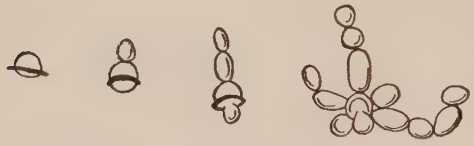


FIG. 43. — The yeast genus Willia. Germination of a spore. (Adapted from Hansen.)

Saccharomycodes. — This genus includes those forms of yeast in which the germinating spore produces a promycellium; that is, the first cell formed after germination is not abstricted as in other yeasts, but is separated from the mother cell by a cross wall. From this cell the usual buds are then formed (Fig. 42). Two species, both unimportant, have been described.

Saccharomycopsis. — This genus resembles Saccharomyces closely except in possessing two spore walls or membranes (Fig. 42). Two unimportant species are known.

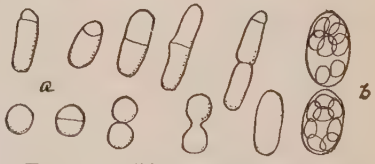


FIG. 44. — The yeast genus Schizosaccharomyces. *a*, various stages in the vegetative multiplication of the cells by fission; *b*, asci and ascospores. (Adapted from Jörgensen.)

Pichia and Willia. — These genera resemble each other and differ from other yeasts in the prompt formation of a film on sugar media. Willia has lemon-shaped, pointed spores; Pichia has round or irregular spores. The latter genus has some eight or

more described species, few of them of importance. Willia (Fig. 43) has seven described species, all unimportant.

Schizosaccharomyces. — This genus is easily differentiated because of its vegetative multiplication of cells by fission and not by budding. Three species have been described; one (*Schizosaccharomyces octosporus* Fig. 44) is the active agent in the fermentation of an African beer.

CHAPTER VII

MORPHOLOGY OF THE MOLDS

The molds are multicellular. In this they differ from the unicellular yeasts and bacteria. This type of plant body renders the discussion of morphology more complex than with bacteria and yeasts, for it is necessary to consider not only the individual cells, but also the manner in which they are combined to make up the mold body and are differentiated into the several types found in the various mold organs. Several hundred genera of molds have been described, contrasting with the somewhat smaller number among the bacteria and the yeasts.

Plant Body of the Mold. — Molds, as well as most other fungi, are made up of more or less branched threads or *hyphæ*, usually consisting of chains of more or less cylindrical cells united end to end. These hyphæ are differentiated on the basis of function into two types, some serve the purpose of securing nutriment from the material in which they are growing, and are called *vegetative hyphæ;* others may produce spores, and are said to be *fertile hyphæ.* The whole mass of vegetative hyphæ, the entire plant body of the mold, is called the *mycelium.* Molds may be differentiated into two types on the basis of the kind of mycelium produced. The hyphæ in the one case are *septate,* in the other *non-septate.* Septate hyphæ are divided at intervals by cross walls or *septa;* that is, the thread is divided into distinct cells (Fig. 45). In the non-septate type cross walls are wholly absent or occur only occasionally, the whole mycelium being thus made up of a more or less branched continuous tube containing protoplasm (Fig. 45). This latter condition is sometimes described, though not accurately, by saying that the entire plant body is a single cell. In the molds having the septate mycelia each cell contains

a single nucleus. A division of the nucleus is followed by the formation of a cross wall separating the two cells. In the non-septate type, on the other hand, there are numerous nuclei embedded in the protoplasm, and the division of a nucleus is not followed by the formation of a septum. Since a cell may be defined as a nucleus surrounded by a bit of cytoplasm, such a mycelium may be regarded as multicellular even though each cell does not have its distinct cell wall. The term *coenocyte* is sometimes used

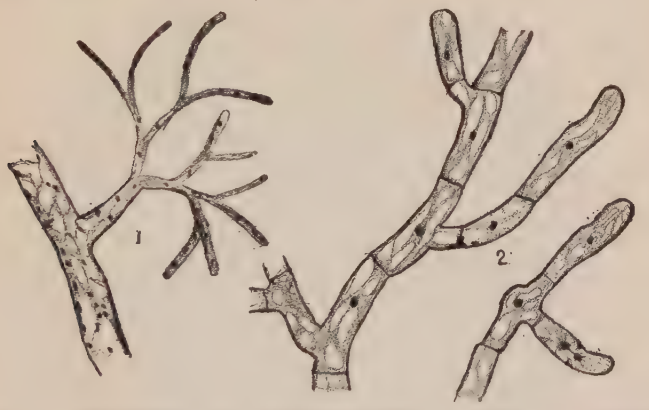


FIG. 45. — Non-septate and septate mycelia of molds. 1, Non-septate mycelium of the Mucor: note the large number of nuclei, the total absence of septa, and the vacuolate nature of the cytoplasm; 2, Septate mycelium of the mold Aspergillus: note the presence of a single nucleus in a cell, the septa and the vacuoles.

to indicate this type of structure. Whether or not the mycelium of a mold is coenocytic or septate is an important factor in mold classification, *i.e.*, the two principal groups of molds may be differentiated by this means.

The structure of the cells of a hypha does not differ materially from that of the yeast cell. There is present the cell wall and the protoplasm made up of ectoplast, cytoplasm, and nucleus. The cells frequently contain vacuoles, granules, oil globules, and sometimes other inclusions. The cell wall is made up in some instances of a substance that gives the reactions characteristic of the cellulose in higher plants; most frequently, however, it consists of a

nitrogenous material resembling chitin. These walls show resemblances in composition therefore to the cell walls of the bacteria.

Growth of a Mold. — All multicellular organisms grow in two ways, by an increase in size of the component cells and by an increase in numbers of these cells. The increase in number of cells may be either *intercalary* or *apical*. In molds showing the former any cell may divide, each cell then increasing in size, frequently

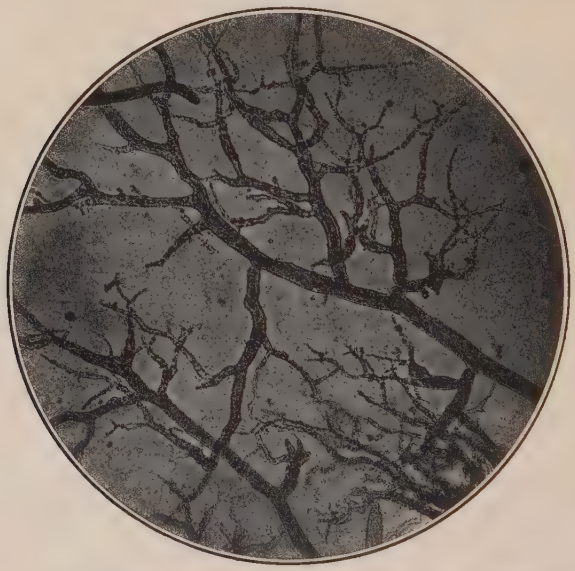


FIG. 46. — Non-septate mycelium of Mucor. (Photomicrograph, × 500.)

with some consequent distortion of the thread. Such intercalary multiplication of cells is relatively rare. In apical growth, by far the commoner type, the terminal cell alone divides, and the filament grows in length only at its tip. Branching of a hypha is due to the division of a cell nucleus followed by a budding out of a lateral filament from the cell.

Reproduction of Molds. — Molds reproduce usually by means of spores, each organism producing these spores in very great numbers. Spore production of molds, therefore, unlike that of the bacteria, is a method of *multiplication* as well as of *reproduc-*

tion. Like those of bacteria and yeasts, the spores of the molds are useful in carrying the organism through unfavorable conditions, and aid in its dissemination.

Mold spores are of two types, sexual and asexual in origin. A *sexual spore* is one that is either the immediate or the indirect product of the fusion of two cells, called *sex cells* or *gametes*. An

FIG. 47. — Septate mycelium of Aspergillus growing on an agar plate culture. (Photomicrograph, × 500.)

asexual spore is one that is produced without the intermediation of sex cells. Practically all molds produce asexual spores. In some cases two or three kinds may be developed by a single species; a few are known to produce sexual spores as well.

Sexual Spores of Molds. — Two principal types of sexual spores occur among the molds, one type in the forms having coenocytic mycelium and another in those with septate hyphæ. These are termed *zygospores* and *ascospores* respectively.

Zygospore formation in Mucor may serve as an illustration of this type. Two cells approximately equal in size rise from filaments

lying close together, enlarge and bend toward each other. Their tips are separated from the remainder of the branch by the formation of cell walls. Finally the tips meet, the walls in contact dissolve, and the contents of the two cells flow together and fuse to form the zygospore (yoke spore) (Fig. 48). A heavy membrane is formed about this cell which then goes into the resting stage. Under favorable conditions it will germinate and reproduce the mold (Fig. 49). Zygospores, as has been noted, occur only on those forms having no cross walls, and are relatively rare even in them.

FIG. 48. — Formation of the Zygospores of a Mucor. 1, two filaments approaching each other; 2, terminal cells separated from parent hyphæ and in contact; 3, cells fusing and enlarging; 4, ripe zygospore with suspensors. (Adapted from Brefeld.)

Ascospore formation does not follow as closely after the union of two cells or gametes as is the case with the zygospore. A number of the genera included in the group of molds having septate mycelia are known to produce ascospores at some time during the life history; probably many others will be shown to produce them also when their complete life cycles have been studied. In this type of spore formation two cells, of the same or different sizes, arise on the same or on adjoining hyphæ. They coil together, the tips fuse, and the contents of one cell pass to the other. Instead of developing immediately into a spore, this fertilized cell grows into a more or less dense mass of branching threads, certain

FIG. 49. — Germination of a zygospore. (Adapted from Tavel.)

cells of which develop into *spore sacs* or *asci*. In each one of these asci there is produced from two to many spores, the usual number being eight. Frequently the hyphæ lying near the sex cells branch and rebranch to form a covering for the mass of asci. Such a structure is called a *perithecium* (Fig. 50). Very few of the genera of molds commonly encountered in the home or in the laboratory produce such perithecia, the principal one being Aspergillus. The botanist makes considerable use of perithecia and their contents in

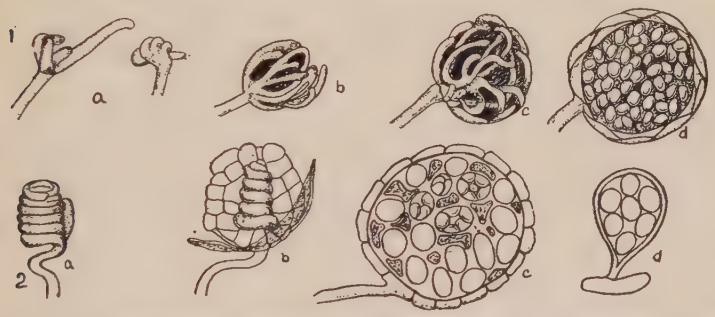


FIG. 50. — Formation of ascospores and asci in molds. 1, Monascus: *a*, sex cells on hyphæ; *b*, *c*, formation of the perithecium by growth of sterile hyphæ; *d*, section through a mature perithecium showing the spores. The latter have escaped from the asci and lie free in the perithecium. 2, Aspergillus: *a*, *b*, *c*, stages in the development of the perithecium; *d*, ascus with ascospores from the interior of the perithecium. (1, adapted in part from Harz; 2, adapted from DeBary.)

formulating logical classifications of the fungi, but they are of little value in the separation of the forms that are of the greatest economic importance.

Asexual Spores of Molds. — Practically all of the molds bear asexual spores. The manner in which these are produced, their size, shape, color, and septation are our most important guides in the determination and the differentiation of genera and species.

Asexual spores are either borne free on the sides or ends of hyphal threads, or they are produced in a specialized spore case called a *sporangium*. A spore not borne in a sporangium is commonly called a *conidium*. Sometimes the same mold may produce two, three, or even four types of asexual spores, rendering classification somewhat difficult.

In a few molds the spores are produced by a segmentation of the mycelium, the hyphæ breaking up into conidia called *oidia* (Fig. 51). Not infrequently cells or sections of a hyphæ may be

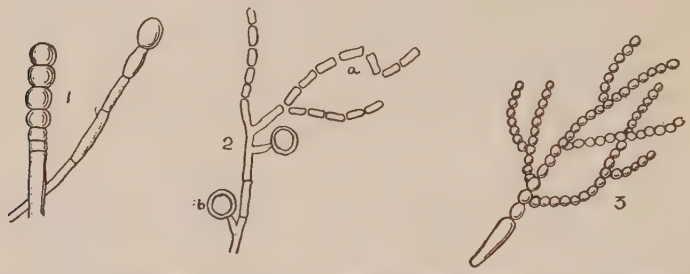


Fig. 51. — Formation of oidia. 1, oidia from Monascus; 2, Endomyces, with oidia (*a*), and chlamydospores (*b*); 3, oidia formation by the mycelium of *Mucor racemosus*. (2 and 3, adapted from Brefeld.)

surrounded by heavy walls; these are termed *chlamydospores* (Fig. 52). They differ from oidia in the possession of a heavy membrane and in that the entire hypha is not broken up into spores. In some other molds the conidia are produced directly on the sides or ends of hyphæ which differ in no way from vegetative hyphæ.

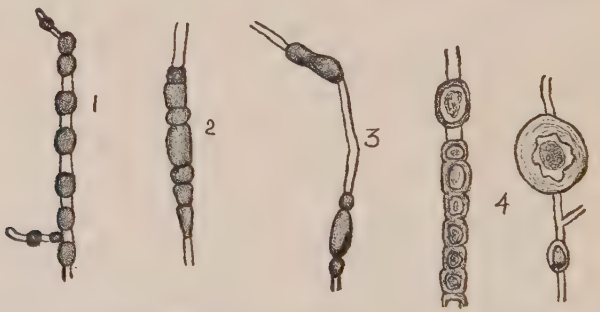


Fig. 52. — Chlamydospores of various molds. 1, *Mucor racemosus;* 2, *Mucor rouxii;* 3, *Rhizopus oryzæ;* 4, *Mucor oryzæ*. (1, adapted from Brefeld; 2 and 4, from Wehmer; and 3, from Went and Geerlings.)

Usually, however, they are borne on specially differentiated fertile hyphæ. A stalk of this type bearing conidia is termed a *conidiophore* (Fig. 53), one bearing a sporangium a *sporangiophore*. The

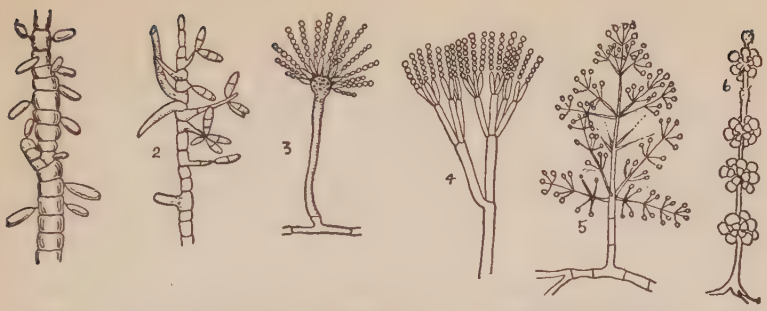


FIG. 53. — Various types of conidiophores. 1, Mülleriella, a form in which conidiophores are absent, the conidia arising laterally directly from the mycelium; 2, Urospora, a form with simple conidiophores, little differentiated; 3, Aspergillus, with enlarged tip of well-differentiated conidiophore, and conidia arising in chains from the sterigmata; 4, Penicillium, with much-branched conidiophore; 5, Acrostalagmus, with conidiophores branched in whorls; 6, Arthrobotrys, simple conidiophore with spores borne in clusters from the enlarged nodes. (1, 2, and 4, adapted from Brefeld; 3, from Kny; 5 and 6 from Corda.)

grouping, size, shape, and branching of the conidiophores and sporangiophores are important in the classification of genera.

Mold conidia may be produced singly or in chains. In the latter case the chains may be simple or branched. Usually a coni-

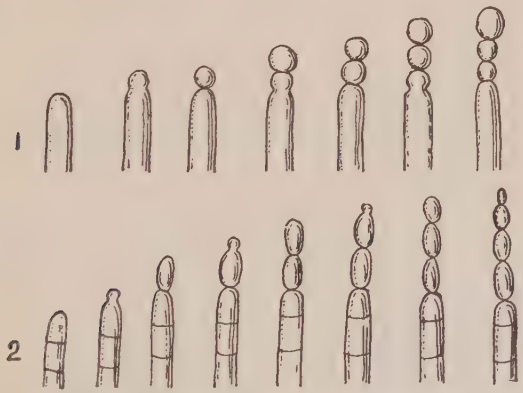


FIG. 54. — Two types of conidial development in molds. 1, Conidia produced by successive abstrictions of the tip of the conidiophore; 2, Conidia formed by the repeated budding of a new conidium from the terminal cell of the chain. (Adapted from Zopf.)

dium is formed by the slight elongation of the conidophore followed by the formation of a cell wall and by an abstriction of the spore. The conidiophore then again elongates and pinches off another spore. It is evident that in this type the youngest spore is the one at the base of the chain (Fig. 54). In a few cases the chain is formed by the repeated division or budding of the terminal cell. In this type the youngest spore is the one at the tip of the chain,

FIG. 55. — Longitudinal sections through sporangia of Rhizopus at various stages of growth. 1, Sporangiophore tip swollen, protoplasm vacuolate, nuclei more numerous toward the periphery; 2, a wedge-shaped section from young sporangium at a little later stage than 1; the sporangial wall is differentiated at *a*, walls are cutting off groups of nuclei from each other to form spores at *b*, and the beginning of the wall of the columella is visible at *c*; 3, similar to 2, but at a later stage in development; spores well separated from each other at *a*, columella wall distinct; 4, mature sporangium, showing the columella (*a*), apophysis (*b*), sporangiophore (*c*), and spores (*d*) of various sizes. (Adapted from Blakeslee.)

i.e., the apical spore. Branching of the chain of spores may occur through the lateral budding of cells (as in Hormodendrum. See Fig. 70).

Sporangia are produced only by molds having a non-septate or coenocytic mycelium, though conidia are also produced by some of the members of this group. A sporangium originates as a terminal swelling of the sporangiophore or one of its branches; this enlargement is separated from the sporangiophore by the formation of a septum and enlarges considerably. The nuclei contained

within it increase in number, and each surrounds itself by a bit of cytoplasm (Fig. 55). This is enveloped in a membrane, and spores are thus formed. The interior of the sporangium is in this manner

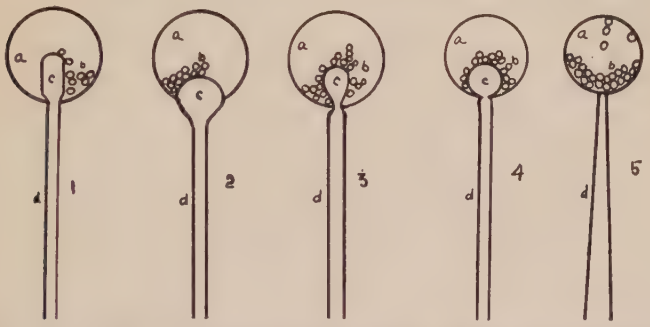


FIG. 56. — Types of sporangia with spores, diagrammatic longitudinal sections. 1, *Mucor mucedo;* 2, *Rhizopus nigricans;* 3, *Mucor racemosus;* 4, *Mucor erectus;* 5, *Mortierella* sp: *a*, sporangium; *b*, spores; *c*, columella; *d*, sporangiophore.

more or less compactly filled with spores. Usually a structure known as a *columella* is also to be found within the sporangium (Fig. 56). This is a more or less club-shaped or hemispherical

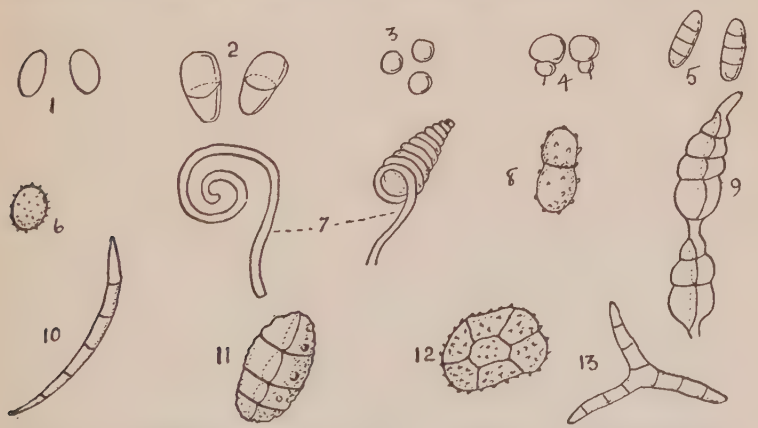


FIG. 57. — Different types of conidia. 1, Oöspora; 2, Trichothecium; 3, Aspergillus; 4, Mycogone; 5, Septocylindrium; 6, Œdocephalum; 7, Helicomyces; 8, Trichocladium; 9, Alternaria; 10, Fusarium; 11, Macrosporium; 12, Epicoccum; 13, Trinacrium.

extension of the sporangiophore into the cavity of the sporangium itself. The other wall of the sporangium is usually a thin membrane which breaks open readily when the sporangium is mature, although in a few types the wall is thick and persistent.

Conidia are of varied shapes: spherical, oval, cylindric, fusiform (spindle shaped), club shaped, irregular, star shaped, or greatly elongated and thread shaped. They may be unicellular or multicellular (septate) (Fig. 57). The septa may be only cross partitions, or they may be longitudinal as well. A conidium divided into a number of cells by walls in different directions is said to be *muriform*.

Spores are much more resistant to unfavorable environment, such as heat, light, drying, and chemicals, than the molds that produce them. Many are well adapted for transportation by currents of air. When brought under favorable conditions for growth, they germinate and reproduce the vegetative mycelium.

CHAPTER VIII

CLASSIFICATION OF THE MOLDS

Limitation of the Term Mold. — It is difficult, if not impossible, to formulate a wholly satisfactory definition for the term *mold*. It should be noted that the systematic botanist does not recognize any such group of fungi. The only justification for the use of the term is an economic one, for the group contains many forms that are of importance in fermentation and in the preservation of food stuffs and other organic materials. It is a group made up of fungi having certain superficial resemblances. All of them are alike in possessing a plant body made up of hyphæ; most of them grow on a variety of dead organic matter, frequently producing fermentation or decay, and a very few cause disease in animals. Many diseases of plants are also caused by closely related forms, but may be excluded from consideration here, for they are of chief interest to the plant pathologist. In short, the group to be discussed is fairly well defined by the common conception of a mold as a more or less cottony, cobwebby, velvety, or powdery organism occurring on decaying organic matter.

Botanical Relationships of the Molds. — Organisms answering to the above general characterization of molds are found in three of the four main subdivisions of the fungi, namely, the *Phycomycetes*, *Ascomycetes*, and *Fungi Imperfecti* (possibly the fourth, the *Basidiomycetes*, should be included also). A brief discussion of these groups follows:

Phycomycetes. — These fungi are distinguished by possession of a non-septate or little septate mycelium and by producing sexual spores directly as the result of the union of two gametes. Usually asexual spores are produced as well; frequently in sporangia, more rarely as conidia. Of the families belonging to the

Phycomycetes, one only, the *Mucoraceæ*, contains organisms usually included with the molds. The commonest of these is the black, bread mold (Rhizopus).

Ascomycetes. — The Ascomycetes are differentiated by the development of the cell resulting from fertilization into a group or mass of cells, some of which become spore sacs or asci, each containing typically eight spores. Usually asexual spores (conidia) are produced as well. In many species the formation of asci and ascospores is of exceptional occurrence, multiplication being by means of the conidia. As will be noted later, probably many forms have altogether lost the power of ascospore production and reproduce asexually exclusively. Several of the commonest of the mold genera belong to the Ascomycetes, such as the green and blue-green molds of oranges, apples, bread, etc.

Fig. 58. — A portion of the hymenium (outer layer of a gill) of a mushroom, showing a cluster of basidia typical of a Basidiomycete with the four terminal spores, borne on sterigmata.

Basidiomycetes. — All produce typical spore-bearing structures called basidia (Fig. 58). Few of these forms would ever be mistaken for molds; they are for the most part the rusts, smuts, puff-balls, mushrooms, and toadstools. None of them will be discussed here.

Fungi Imperfecti. — All fungi that do not fall into one of the three preceding groups are placed among the *Fungi Imperfecti*. Probably in most cases they are Ascomycetes whose perfect or ascus stages have never been found or that have wholly lost the ability to produce ascospores. Most of the molds to be considered belong here.

Classification of the Molds. — The molds are sometimes grouped together for convenience and called *Hyphomycetes*, ranking with the classes noted above. It is evident that this is not in strict accordance with relationships, and does not represent an evolutionary classification. It possesses the advantage, however, of facilitating the construction of a key which renders identification of the various mold genera comparatively simple.

Several hundred genera and thousands of species of Hyphomycetes have been described, scattered among five families. Com-

paratively few of these genera, however, include the great majority of all the molds commonly met in the home and in the laboratory. It is of interest to know what characters are used in telling the different kinds apart. These are the various morphological characters which have previously been enumerated. A key which will permit the identification of the genus of any of the commoner molds is included in the appendix. The following key will enable the determination of the genus in most cases. This key is based wholly upon differences in asexual production of spores. It is followed by a more detailed description of the genera, and in some cases of certain important species. These descriptions of the genera should be consulted before assuming the accuracy of a determination made by this key. If the mold studied does not agree, use the key in the appendix.

Key to the Families and Genera of the Hyphomycetes or Molds

(Including only the thirteen most common genera)

a. Family I. **Mucoraceæ.** Spores borne in sporangia

b. Sporangiophores arising in clusters from nodes or runners or stolons of the mycelium . **Rhizopus**

bb. Sporangiophores arising singly, without stolons . **Mucor**

aa. Spores (conidia) never borne in sporangia (true Hyphomycetes)

b. Conidiophores not united into definite masses, usually separate and distinct

c. Family II. **Mucedinaceæ.** Neither hyphæ nor conidia smoky or dark in color

d. Conidia one celled

e. Conidia formed by segmentation of hyphæ, without distinct conidiophores (oidia) . **Oöspora (Oidium)**

ee. Conidia formed on well-differentiated conidiophores. In chains

f. Conidiophores inflated at apex . . . . . **Aspergillus**

ff. Conidiophores branched, forming a brush-like mass **Penicillium**

dd. Conidia two celled, pear shaped
. . . . . **Trichothecium**

cc. Family III. **Dematiaceæ.** Either hyphæ or conidia dark or smoky, frequently both

d. Conidia one celled and on distinct conidiophores. Conidiophore with branches at tip and branched chains of conidia
. . . . . **Hormodendrum**

dd. Resembling Hormodendrum but spores becoming two celled in old cultures
. . . . . **Cladosporium**

ddd. Conidia many celled, muriform, in chains
. . . . . **Alternaria**

bb. Conidiophores united into definite masses

c. Family IV. **Stilbaceæ.** Conidiophores united into a stalk or bundle

d. Hyphæ and spores not dark and smoky **Isaria**

dd. Hyphæ and spores dark or smoky **Stysanus**

cc. Family V. **Tuberculariaceæ.** Conidiophores in a more or less flattened mass or stratum. Molds belonging to this family uncommon. See Appendix.

Family I. Mucoraceæ. — As has been previously noted, this family belongs among the Phycomycetes. Botanically it is characterized by the formation of *zygospores*. These zygospores are not commonly produced by most species, hence the asexual spores and their arrangement furnish a somewhat simpler and more useful means of classification. Most of the genera produce sporangia; a few do not, asexual reproduction then being by means of conidia. About eighteen genera are known. The two most important are the black molds, *Rhizopus* and *Mucor*.

Rhizopus. — Species belonging to this genus are commonly found on decaying vegetables and on bread. The spores are usually present in the laboratory air. The vegetative mycelium penetrates the material on which the mold is growing and soon sends up into the air long, slender threads known as aërial hyphæ. These

grow until they come in contact with some solid object such as the walls of the vessel; they then produce clusters of rootlike holdfasts or *rhizoids* (Fig. 59). These are plainly visible through the glass sides of a flask or a petri dish in which the mold is growing. A thread which attaches itself in this fashion often continues to grow and again throws out rhizoids. On account of its resemblance to a strawberry runner, such a thread is termed a *stolon*. From each of the points where the rhizoids are produced, there develops a cluster of unbranched sporangiophores. The sporangium which develops at the tip of each contains a large number of spores. When

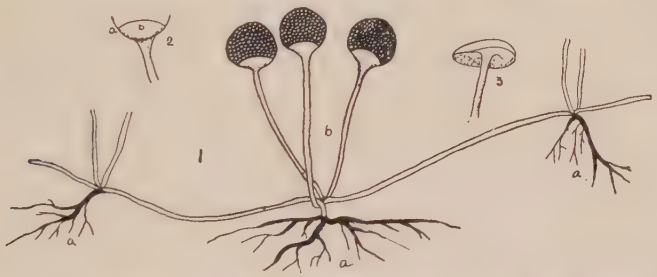


FIG. 59. — *Rhizopus nigricans*. 1, growth habit of the mold, showing the stolons, the rhizoids (*a*), cluster of sporangiophores (*b*), with sporangia filled with spores; 2, lower portion of a sporangium to show the apophysis (*a*), and columella (*b*); 3, lower portion of sporangium with a collapsed columella. When mature sporangia are examined microscopically, particularly when mounted in water, the columella collapses in this fashion.

young it is white, but soon turns black, due to the ripening of the spores. A columella is present but is through part of its length united with the sporangium wall. The sporangium wall breaks to pieces readily when mature and releases the spores. The columella frequently collapses when mounted in water for examination, the lower half of the hemisphere invaginates, and the whole assumes the appearance of an open umbrella with the sporangiophore as the handle (Fig. 59, *3*).

To the naked eye Rhizopus is a somewhat coarse fuzzy or cottony mold, frequently growing over a considerable area of the substrate. When the sporangia have formed it becomes grayish due to the presence of great numbers of these black spheres just visible to the eye.

The most common mold of this genus is the *Rhizopus nigricans*, the black bread mold. It is found on all sorts of decaying vegetables, fruits, and meals. *Rhizopus oryzæ*, *R. japonicus*, and several other species have been described from fermenting meals and grains. Some will be discussed later as of importance in the manufacture of industrial alcohol.

Mucor. — Mucors are also common on decaying vegetables, fruit, bread, etc. This genus differs from the preceding principally in that there are no stolons produced. The sporangiophores do not arise in clusters but are produced singly. In some species they

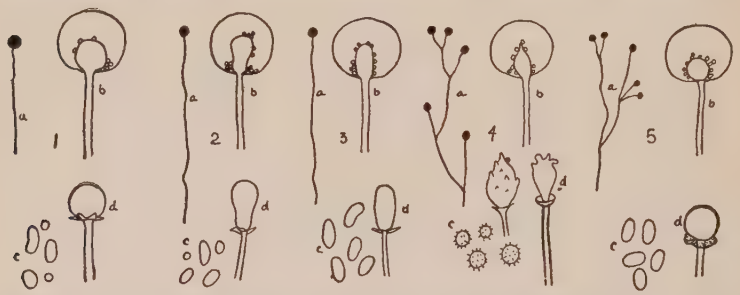


FIG. 60. — The principal species of Mucor. *a*, sporangiophore and sporangium (or sporangia); *b*, single sporangium in longitudinal section; *c*, spores; *d*, columella with sporangium wall fallen away. 1, *Mucor hiemalis;* 2, *M. piriformis;* 3, *M. mucedo;* 4, *M. plumbeus;* 5, *M. rouxii;* (1, 2, 3, and 5, adapted from Wehmer; 4, from Gayon).

branch. The sporangium resembles that of the Rhizopus closely, the columella is usually not as large, and the sporangium wall is frequently somewhat more resistant. To the naked eye a Mucor like Rhizopus is a somewhat coarse, cottony mold. The different species are distinguished from each other largely by branched or unbranched sporangiophores, shape of columella, and size and shape of sporangium and spores (Figs. 60 and 61). Some of the species of Mucors produce heavy-walled resting cells or *chlamydospores* in the vegetative mycelium (*Mucor racemosus*). One of the Mucors (*M. rouxii*) has been used in the saccharification of starch for the purpose of alcohol production. The accompanying key [1] will assist in the differentiation of the commoner and more important species.

Family II. Mucedinaceæ. — This family is one of the subdivisions of the *Fungi Imperfecti*, and as here considered contains some ascomycetous genera. The spores or conidia are never borne

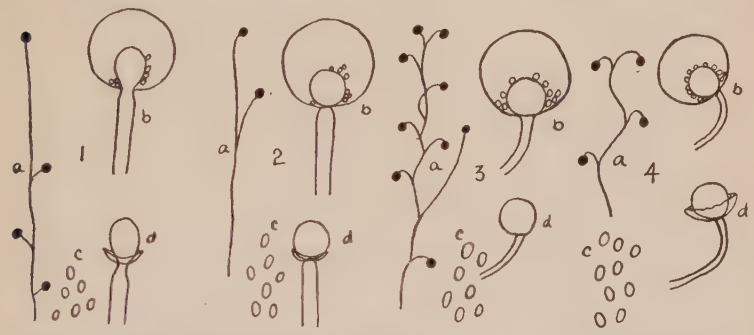


FIG. 61. — The principal species of Mucor, continued. *a*, sporangiophore and sporangia; *b*, longitudinal section through a sporangium; *c*, spores; *d*, columella with the sporangial wall fallen away. 1, *Mucor racemosus;* 2, *M. erectus;* 3, *M. circinelloides;* 4, *M. alternans;* (1, adapted from Riess; 2, from Bainier; 3, from Gayon and Subourg; 4, from Gayon).

in sporangia, and neither they nor the hyphæ are ever dark or smoky. About fifty well-authenticated genera have been described. Four at least of these are quite common.

[1] KEY TO SOME OF THE BEST KNOWN SPECIES OF MUCOR

I. Sporangiophores rarely or never branched
  A. Columella spherical . . . . . *Mucor hiemalis*
  B. Columella pear shaped (piriform) . . . . . *M. piriformis*
  C. Columella elongate to ellipsoidal . . . . . *M. mucedo*
II. Sporangiophores usually branched
  A. Columella usually knobbed or spiny near tip . . . . . *M. plumbeus*
  B. Columella not roughened at tip
    1. Rarely fruiting at room temperatures, important in commercial alcohol manufacture, rapidly saccharifying starch . . . . . *M. rouxii*
    2. Readily fruiting at room temperature, not commercial types
      a. Sporangiophores with definite main stem and secondary lateral branches, racemose
        (1) Columella ovoid . . . . . *M. racemosus*
        (2) Columella spherical
          (*a*) Sporangium gray yellow . . . . . *M. erectus*
          (*b*) Sporangium black . . . . . *M. fragilis*
      b. Branches of sporangiophore nearly equal, cymose
        (1) Sporangia borne irregularly . . . . . *M. ambiguus*
        (2) Sporangia in two rows, alternating
          (*a*) Spores spherical to short ellipsoidal . . . . *M. circinelloides*
          (*b*) Spores longer, ellipsoidal . . . . . *M. alternans*

Oöspora (Oidium). — The designation most frequently used for this genus is Oidium, but the botanist reserves this name for an entirely distinct fungus and substitutes Oöspora as the correct term. One species is of considerable importance, the *Oöspora* (or *Oidium*) *lactis* found commonly on soured milk, in cheese, and in other milk products (Fig. 62). It may also develop in nutrient gelatin exposed to the air. The mycelium of this fungus grows almost entirely within the nutrient material, but chains of spores formed by the segmentation of aërial hyphæ may project above the surface. Unlike most of the other common molds, the vegetative mycelium itself also frequently segments into conidia or oidia. This spore formation within the substratum or food material in which the mold is growing is quite characteristic. The branching of the mycelial threads is also somewhat unusual; they fork into two equal branches, instead of one continuing as a main thread and the other as a lateral offshoot. This type of branching continuously into twos is termed *dichotomous*.

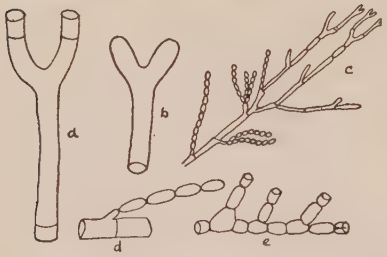


FIG. 62. — *Oöspora* (*Oidium*) *lactis*. *a*, *b*, showing method of branching; *c*, *d*, *e*, method of spore or oidium formation. (Adapted from Thom. Bull. 82, U. S. Dept. of Agr. Bureau of Animal Industry.)

Aspergillus. — Representatives of this genus are common upon decaying vegetables, moldy corn, and grain, as greenish, yellow, orange, brown, or black, velvety or powdery molds. The spores are common in the air and may develop in imperfectly sterilized laboratory media or in media that have been exposed to dust contamination. This genus belongs among the Ascomycetes, for many species produce ascospores. The asci and ascospores are borne in spherical golden yellow perithecia, usually lying closely appressed to the vegetative mycelium. The characteristic method of bearing conidia renders recognition of this genus relatively easy. The conidiophores are unbranched and usually long. They are swollen or enlarged at the tip, and upon the surface there appear

numerous short stalks, usually set close together, giving to the conidiophore the appearance of a war club with spikes. These small stalks are termed *sterigmata* (sing. *sterigma*). They may be simple or branched; if the latter, the mold is sometimes given the generic name of *Sterigmatocystis*. From the tips of these sterigmata chains of spores are abjointed. These chains under favorable conditions may develop to considerable length. Many species of Aspergillus have been described. The species are separated into groups on the basis of the color of the mature spore masses. The key[1] to the commoner species is given below. A large number of species beside those listed have been described (Figs. 63 and 64). The classifi-

FIG. 63. — Hyalopus. Conidiophores with conidia embedded in a globule of mucus.

[1] KEY TO COMMON SPECIES OF ASPERGILLUS

I. Spores white or nearly so
  A. With unbranched sterigmata . . . . . *Aspergillus candidus*
  B. With branched sterigmata . . . . . *A. albus*
II. Spores colored
  A. Spores green, grayish, bluish or yellowish green
    1. With unbranched sterigmata
      a. Producing perithecia readily
        (1) Peritheciun naked, not embedded . . . . . *A. glaucus*
        (2) Perithecium embedded
          (*a*) Tip of conidiophores only slightly swollen, club shaped, sterigmata along sides for a considerable distance . *A. clavatus*
          (*b*) Tip of conidiophore hemispherical, sterigmata produced only from terminal portion . . . . . *A. fumigatus*
      b. Not producing perithecia (so far as known)
        (1) Tip of conidiophore, very large, elongate 80 — 100 μ × 500 — 800 μ . . . . . *A. giganteus*
        (2) Tip of conidiophore smaller, spherical, or hemispherical
          (*a*) Conidiophore rough, warty . . . . . *A. flavus*
          (*b*) Conidiophore smoother . . . . . *A. oryzæ*
    2. With branched sterigmata
      a. Mycelium rusty brown . . . . . *A. versicolor*
      b. Mycelium not so
        (1) Tip of conidiophore club-shaped, sterigmata both lateral and terminal . . . . . *A. pseudoclavatus*
        (2) Tip of conidiophore hemispherical, sterigmata terminal *A. nidulans*
  B. Spores black or dark brown
    1. With unbranched sterigmata . . . . . *A. calyptratus*
    2. With branched sterigmata . . . . . *A. niger*
  C. Spores, yellowish brown, yellow, brown, or reddish
    1. With unbranched sterigmata, coffee-brown spores . . . . *A. wentii*
    2. With branched sterigmata, yellow-brown spores . . . . . *A. ochraceus*

cation of the various species has not been satisfactorily worked out, and many undescribed forms may be isolated.

Of the black spored forms, *Aspergillus niger* is of importance because of its active fermentative ability in sugar solution. It produces oxalic acid and brings about many other changes. It has

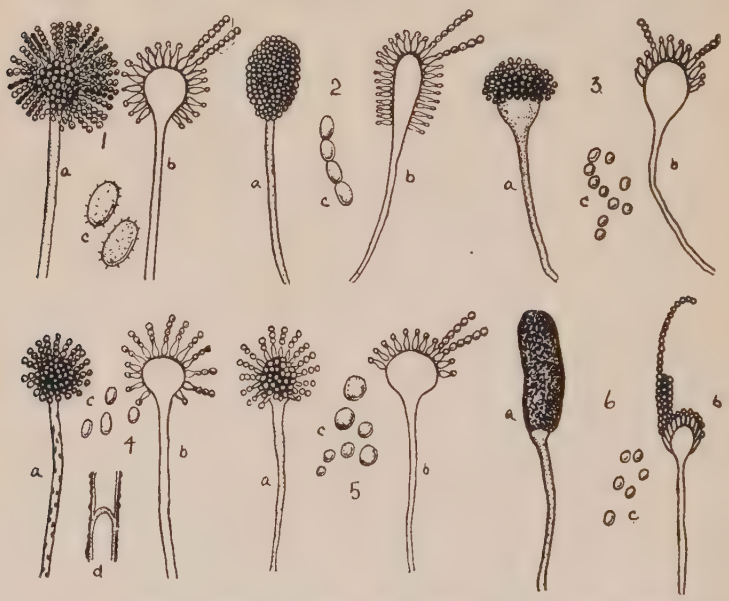


FIG. 64. — Principal species of the genus Aspergillus. *a*, conidiophore with conidia; *b*, longitudinal section through conidial head showing the swollen tip of the conidiophore and the attachment of the conidial chains; *c*, conidia; *d*, section of conidiophore to show roughening. 1, *Aspergillus glaucus;* 2, *A. clavatus;* 3, *A. fumigatus;* 4, *A. flavus;* 5, *A. oryzæ;* 6, *A. calyptratus.* (6, adapted from Oudemans, remainder from Wehmer.)

been used more than any other mold for the study in the laboratory of mold nutrition, metabolism, and enzyme action. *A. glaucus* is one of the commonest of the green spored species on moldy grain, silage, canned fruits, and jellies. It usually produces golden yellow perithecia in abundance in the older cultures. *A. fumigatus* produces a fatal pneumonia in birds when inhaled, and is believed to be pathogenic for animals. The commonest of the white or

cream spored forms is *A. candidus*, of the yellowish green *A. flavus*, and of the yellow *A. ochraceus*. *Aspergillus oryzæ* has been used commercially in the conversion of starch to sugar preliminary to its fermentation by yeast for the production of alcohol.

The genus *Citromyces* is sometimes separated from Aspergillus. In Citromyces the sterigmata are quite long and relatively few (Fig. 66). *Citromyces pfefferianus* produces citric acid when grown in a solution of sugar.

Fig. 65. — Aspergillus, showing conidiophore and head. (Photomicrograph, × 250.)

Penicillium. — This genus is closely related to the preceding and is classed among the Ascomycetes although the formation of perithecia is very rarely observed, and possibly never occurs with many species. The representatives of this genus are probably the most common of all molds. It is the blue-green mold observed on oranges, lemons, apples, and other fruits, vegetables, preserves, grains, hay, in short, on almost any moist organic substance. The genus is differentiated from Aspergillus by the manner in which the spores are borne. The vegetative mycelium penetrates the substratum, and later sends up erect aërial hyphæ as conidiophores. These branch one or more times in whorls, giving rise to a terminal cluster of parallel hyphæ; each of these ultimate branchlets is to be regarded as a sterigma. From each of them a chain of conidia is abstricted. The branches and conidia together resemble a broom

Fig. 66. — Citromyces. 1, conidiophore with sterigmata and conidia; 2 and 2′, hyphæ with crystals of calcium citrate; 3, conidia; 4, crystals of calcium citrate. (Adapted from Wehmer.)

or a camel's-hair brush, hence the name Penicillium (Lat., a little brush) (Fig. 67).

As in Aspergillus, the color of the mature mold is used as a basis of separation of groups of species, but is not altogether reliable as it may vary somewhat with environment. The species of this

Fig. 67. — Common species of the genus Penicillium. *a*, characteristic branching of the conidiophores; *b*, same on a smaller scale; *c*, conidia. 1, *Penicillium expansum;* 2, *P. italicum;* 3, *P. digitatum;* 4, *P. roquefortii;* 5, *P. brevicaule;* 6, *P. camembertii.* (Adapted from Thom.)

group are among the most difficult of fungi to identify and differentiate satisfactorily. Several have been shown to be of importance in the ripening of certain types of cheese, as *Penicillium roquefortii* in Roquefort cheese and *P. camembertii* in Camembert cheese. *P. expansum* is common on many decaying substances, particularly on apples (Fig. 68). When growing under favorable

conditions, considerable numbers of the conidiophores may unite into a white stalk bearing the green spores at the tip. These masses, readily visible to the unaided eye, are termed *coremia*. *P. glaucum* is the name that was first given to a green Penicillium, and is frequently used to indicate any one of this group. Altogether several hundred species of Penicillium have been described.

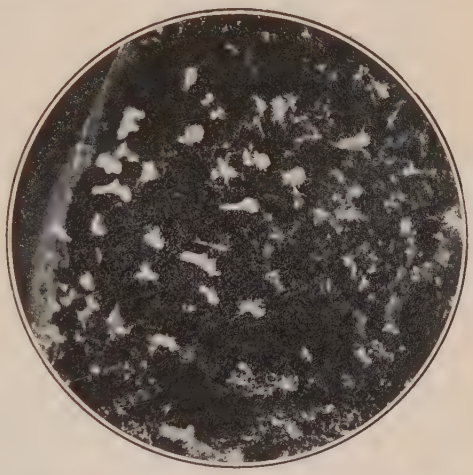


FIG. 68. — A portion of the surface of a moldy apple showing the coremia of *Penicillium expansum* projecting above the surface. (Photomicrograph, × 10.)

Trichothecium (Cephalothecium). — The members of this genus are not uncommon on decaying fruits, particularly apples. The mycelium penetrates the substratum, and sends into the air at intervals straight unbranched conidiophores little or not at all enlarged at the tip. The spores may be solitary or in a loose cluster. The conidia are two celled, one cell frequently being somewhat larger than the other. The most common species is *Trichothecium roseum*. The specific name comes from the faint rose or pink color of the mold, particularly of the spores. This mold is common on the apple and is sometimes the cause of considerable damage through the development of a rot. The spores are not uncommon in the air and develop readily on culture media in the laboratory.

FIG. 69. — Trichothecium. Stages in the development of the conidia. (Adapted from Craig and Van-Hook.)

Family III. Dematiaceæ. — The molds of this family are to be differentiated from the preceding by the presence of a dark, usually

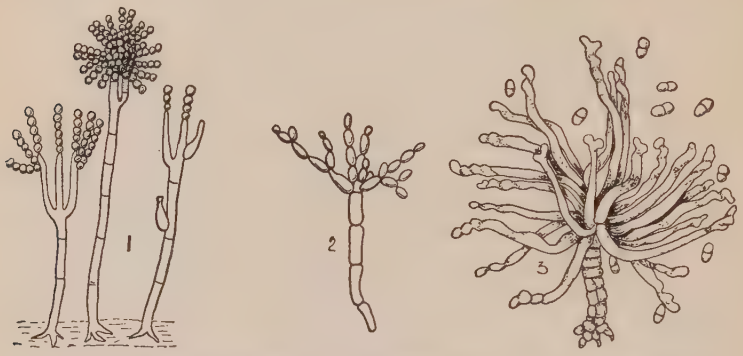


FIG. 70. — 1, Haplographium; 2, Hormodendrum; 3, Cladosporium. (1, adapted from Saccardo; 2, from Bruhne, and 3, from Janczewski.)

brown or smoky, cell wall. The mold colony itself is dark in consequence, sometimes black.

Haplographium, Hormodendrum, and Cladosporium (Fig. 70). — These genera are so closely related and so intergrade into each other that they will be considered together. They are common on decaying paper, wood, fruits, and vegetables, usually producing dark or sooty patches. They are to be recognized by the dark or smoky appearance of the mycelium and usually of the spores as well. The latter are borne in more or less irregular chains. The conidia of Haplographium and Hormodendrum are unicellular. Cladosporium produces two-celled spores, at least in old cultures, but in young cultures the mold closely resembles Hormodendrum. *Cladosporium herbarum* is one of the commonest of molds encountered in the laboratory. In each of these forms the vegetative mycelium

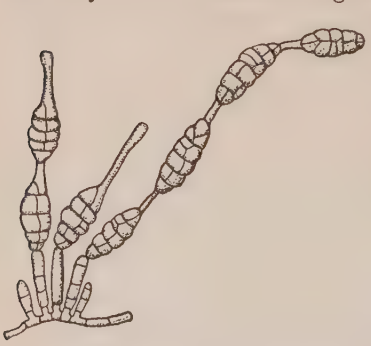


FIG. 71. — Alternaria, conidiophores with chains of muriform conidia.

of brown, much-branched hyphæ penetrates the substratum and sends up more or less well-differentiated conidiophores above the surface.

Alternaria. — The molds belonging to this genus have large, brown, many-celled spores borne in chains on short conidiophores (Figs. 71 and 72). The septa in the spore occur both at right angles and parallel to the long axis, and the spore is said to be *muriform.*

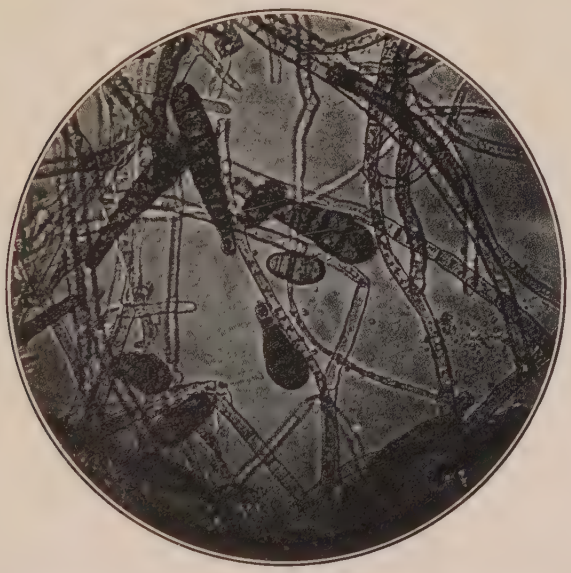


FIG. 72. — Alternaria, showing conidia, conidiophores, and mycelium. (Photomicrograph, × 400.)

Many species of Alternaria have been described from decaying vegetation. The spores are common in the air. The most abundant species is probably *Alternaria tenuis*, found on moldy grain, seeds, leaves, hay, in the soil, and commonly infecting laboratory media. Some species are believed to cause disease in certain plants.

Family IV. Stilbaceæ. — The genera of this family are characterized by a definite bunching or massing of the conidiophores into bundles of parallel threads. These bundles or stalks are termed

coremia. The spores are borne usually at the tips of the hyphæ constituting these coremia, sometimes also along the sides.

Isaria. — This genus is characterized by its lack of brown or smoky pigment in the spores and hyphæ. The spores are borne singly on the sides or tip of the coremium. The latter is usually more or less club shaped or cylindrical, and is sometimes branched. The spores are not grouped in definite heads. Species of Isaria develop not infrequently upon culture media exposed to the air, upon potatoes, and other vegetables (Fig. 73).

Fig. 73. — Isaria. A coremium with terminal conidia on the conidiophores. (Adapted from Saccardo.)

Fig. 74. — Stysanus. A coremium showing terminal chains of conidia on the conidiophores. (Adapted from Saccardo.)

Stysanus. — This genus differs from Isaria in the dark color of the spores and hyphæ. The coremia bear spores usually only near the tip. The spores are produced in chains. The commonest species is *Stysanus stemonitis*, which is abundant upon decaying vegetation everywhere, and often develops on media exposed to the laboratory air (Fig. 74).

Family V. Tuberculariaceæ. — Only a few of the genera of this family are to be included among the molds. The greater number are plant parasites. This family is differentiated by the formation of a dense mass of branched hyphæ, called a *sporodochium*, from which the spores are produced. Molds of this group are not common, and none of the genera will be described here.

CHAPTER IX

DISTRIBUTION OF MICROÖRGANISMS

Microörganisms are not found at great altitudes in the air, at considerable depths in the soil, nor in the healthy normal tissues of animals and plants. Otherwise they are ubiquitous wherever the temperature is not so high as to destroy life. They are abundant in the soil and air, in most surface water, in certain foods, in decaying organic matter of all kinds, on the skin, and within the intestines of man and animals.

Microörganisms of the Soil. — Bacteria are very abundant in most soils. A rich garden soil will contain from a hundred thousand to as many million per gram. In acid moor or peat soils, in soils containing an excess of alkali, and in poor sandy soils they are much less numerous. These bacteria perform many functions in the soil, fitting it physically and chemically for the growth of higher plants. Except for the soil bacteria plant life could not long persist. They decompose organic matter directly and the minerals of the soil indirectly, converting these soil constituents into compounds that may be taken up by the roots and assimilated by green plants. Some forms take up atmospheric nitrogen which is ultimately used by other plants. Bacteria capable of producing disease in plants or animals are only occasionally found in the soil.

Molds are also quite abundant in soils. In some, as in those of forests, they may bring about decay even more efficiently than do bacteria. The decomposition of cellulose and woody tissues is in large part the work of molds and related fungi.

Yeasts are relatively infrequent and unimportant in the soil. This does not mean that they are often entirely absent, for they usually may be demonstrated by appropriate cultural methods.

Microörganisms in Water. — Bacteria are commonly present in most waters; yeasts and molds much more rarely. Water from

deep wells, such as the artesian wells of some districts, and from some springs may be entirely free from bacteria. Most well waters contain from a very few to several hundreds of bacteria per cubic centimeter. Lakes and ponds not polluted with sewage have about the same number. Streams usually have more. Sewage and heavily polluted water may contain thousands or millions per cubic centimeter. While water does not normally contain disease-producing bacteria, it may become polluted with the excretions of diseased animals or man, and become a dangerous source of infection.

Microörganisms in Foods. — Food when eaten is rarely free from living organisms. The type of organisms present, whether bacteria, yeasts, or molds, is determined by the chemical composition of the food and by its previous treatment. The organisms of food may be divided into three groups: those that are of benefit in bringing about desirable fermentations, such as that which occurs in the preparation of sauerkraut or dill pickles; those that produce undesirable fermentations and decay, and those that are capable of producing disease. The whole problem of food preservation, including refrigeration, canning, and drying, is that of preventing the growth of undesirable organisms.

Some healthful foods contain great numbers of bacteria. Clabbered milk, for example, may contain hundreds of millions per cubic centimeter. Foods not freshly cooked usually contain them in considerable numbers, but they are generally not of types that are injurious. Occasionally pathogenic bacteria may be present in the meat of diseased animals or in other food that has been improperly cared for.

Bacteria are abundant in most foods; yeasts may be present in those containing sugar; molds occur upon many fruits and their products, and are instrumental in the ripening of certain cheeses.

Microörganisms in Fermentation and Decay. — The decomposition of organic matter, of all plant and animal remains, is brought about by the activity of microörganisms. Proteins are usually decomposed by bacteria, although molds are occasionally important. Complex carbohydrates, such as starch and cellulose, are commonly broken down by molds and by a few bacteria; the simpler

carbohydrates are fermented by yeasts, molds, and bacteria, and the fats by bacteria and molds.

Microörganisms of the Body. — Since the body is constantly in contact with substances covered with bacteria, the skin harbors many organisms; and as food is not usually sterile when eaten, the alimentary tract (particularly the intestines) contains bacteria in large numbers. The skin and intestines soon come to possess what may be regarded as a normal flora of bacteria that multiply in these respective situations. These bacteria usually do no harm. If they penetrate the skin or the intestinal wall, the cells and fluids of the body destroy them. Yeasts and molds are much more rarely found under these conditions. The living normal tissues of the body are bacteria free. Pathogenic bacteria, however, may break down the barriers to invasion of the body and produce disease. The body excretions, particularly the feces, contain bacteria in enormous numbers, as much as twenty-five per cent by weight of the latter being bacterial cells.

How Microörganisms are Scattered. — Those bacteria that possess organs of motion can swim to some distance from their origin under suitable conditions. Molds, and to a lesser degree yeasts and bacteria, may spread by direct growth. The mycelium of some molds, for example, may grow at the rate of an inch or more a day. The most common medium of dispersal is the air. Certain of the molds are particularly adapted to distribution by this means. They send up their erect conidiophores away from the moist substratum on which they are growing and produce their conidia where they are readily dislodged and blown about by the slightest air current. Bacteria and yeasts are not readily blown about until the material in which they have been growing has dried and has been pulverized. In consequence the air on a dry day, particularly if windy, contains many more organisms than when moist. Organisms are carried also by water, as that in streams, and in milk and other fluids. Insects, particularly flies, may transport them in considerable numbers from one place to another. The last methods are all of particular importance as being those whereby certain of the disease-producing bacteria are transmitted from one individual to another.

SECTION II

CULTIVATION AND OBSERVATION OF MICROÖRGANISMS

CHAPTER X

STERILIZATION

STERILIZATION may be defined as that process whereby any material is entirely freed from living microörganisms. The fact that microörganisms are present upon the skin, in dust, in the air, upon the surfaces of all laboratory tables and apparatus, renders it evident that means must be taken to prevent such forms from coming in contact or becoming mixed with the organisms which it is desired to study in pure cultures.

Sterilization may be effected either by physical or by chemical means. Destruction of organisms by chemical agencies is frequently designated as disinfection, and the use of the term *sterilization* is confined to their destruction by physical means. The physical agents most commonly employed in sterilization are heat, filtration, and light.

Sterilization by Heat. — Sterilization may be accomplished by momentary heating to a very high temperature (to red heat) or by a longer exposure to hot air, to streaming steam, or to steam under pressure, and for some purposes by the use of temperatures somewhat lower than the boiling point of water.

Sterilization by the Flame. — Small objects that are not easily injured by heat may be sterilized by thrusting them into the flame of the bunsen burner and allowing them to be heated to a temperature high enough certainly to destroy any organisms that may be present upon the surface. This ordinarily means red heat. The platinum needles commonly used in the laboratory in the transfer of bacteria from one culture tube to another are generally sterilized in this manner.

Sterilization by Hot Air. — Dry glassware, such as flasks, test tubes, and similar apparatus, is commonly sterilized by the use of

a hot-air oven (Fig. 75) which can be maintained at a temperature of 150° to 170° C. for an hour. Care must be used to see that objects to be sterilized are not packed into the oven so tightly that heat will not penetrate readily to all parts. This method cannot be used in the sterilization of liquids or of organic substances which may be injured or decomposed by such temperatures.

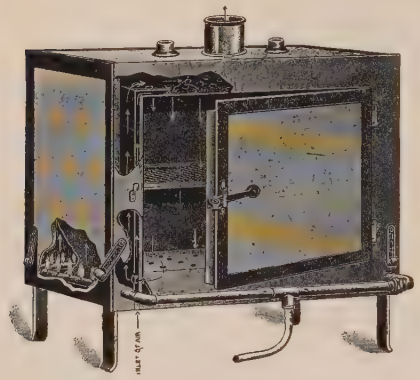


Fig. 75. — Hot-air oven for sterilizing.

Sterilization by Streaming Steam. — Moist heat is far more efficient in sterilization than dry heat. Streaming steam or live steam is therefore quite effective and can be used for organic substances such as the laboratory media. Such sterilization is ordinarily accomplished in an Arnold steam sterilizer or some similar apparatus which allows the live steam to come in contact with the material to be sterilized. An Arnold sterilizer is illustrated in Fig. 76. It may be seen to consist of a pan partially filled with water, a portion of which is between the layers of the double bottom. This water between the bottoms soon reaches boiling temperature when placed over a flame and is replaced through suitable openings from the water in the pan above as rapidly as it evaporates. The steam arises through the large opening in the center and escapes finally through the openings in the top or around the doors. Live

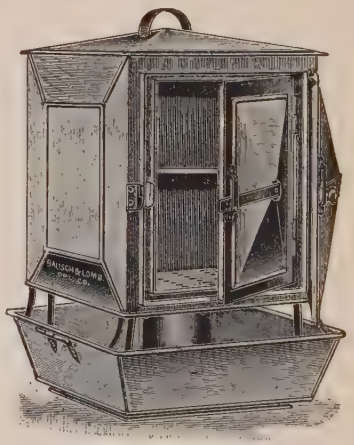


Fig. 76. — Arnold Steam Sterilizer for intermittent sterilization in streaming steam.

steam (at sea level) has a temperature of about 100° C. A single exposure for fifteen minutes to such a temperature is ordinarily sufficient to destroy all vegetative bacteria. Some resistant spores, however, may remain alive after this treatment. During the next few hours, however, they usually germinate, and a second subjection to this process twenty-four hours after the first, will destroy all of these. In practice it is customary to sterilize on three successive days for fifteen minutes on each day. This method of sterilization does not subject the constituents of the medium to a high temperature for a long period of time. This process is called *intermittent sterilization*.

Practically the same method is used for the preservation of foods. Vegetables, for example, are sometimes heated for an hour or more on three or four successive days. Streaming steam and boiling water are commonly used in the complete or partial sterilization of many household and dairy utensils. In the creamery, for example, milk bottles, cans, and other apparatus are subjected to streaming steam or are "scalded" with boiling water.

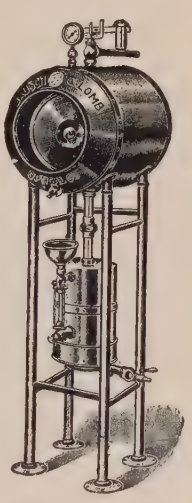


Fig. 77. — Autoclave for sterilizing in live steam under pressure.

Sterilization by Steam under Pressure. — The apparatus most used for sterilization by steam under pressure is called the autoclave or digester (Fig. 77). It consists essentially of a closed chamber into which steam under pressure can be introduced. It is necessary that all of the air originally present in the apparatus shall be driven out by the live steam; therefore the stopcock on the autoclave must be left open until all the air has escaped. The pressure of live steam varies directly as its temperature; consequently when one knows the pressure as indicated by a gauge, the temperature is readily determined. It may, in fact, usually be read from the gauge itself. This is not true, however, of a mixture of steam and air, for such a mixture has a decidedly lower temperature than would be indicated by the pressure. It is customary to sterilize most media at a pressure of fifteen

pounds for ten to fifteen minutes. This pressure gives a temperature of about 121° C. If the material to be sterilized is very bulky (large flasks filled with media, for example) it is necessary to heat for a longer period of time to make sure that the temperature of the medium has been raised to that of the steam. This temperature is capable of certainly destroying any bacteria that may be present. For sterilizing gelatin it is sometimes necessary to decrease the pressure to ten pounds and expose to this temperature for ten minutes only. As has been mentioned above, some organic substances, among them certain of the sugars, cannot be sterilized in the autoclave without decomposition.

The common procedure in the commercial preparation of canned foods, such as corn, tomatoes, peas, beans, etc., is essentially treatment in an autoclave by steam under pressure for varying lengths of time. The pressure cookers are similarly used in preparation and canning of foods in the home.

Fig. 78. — Inspissator, for the sterilization of media at temperatures below the boiling point of water.

Sterilization at Temperatures Lower than Boiling Point. — It is sometimes necessary to use temperatures lower than the boiling point of water in order to prevent undesirable chemical changes in the medium which is being sterilized. Under such conditions heat must be applied for a longer period (for an hour or more) on each of five or more successive days. Commonly a temperature of from 75° to 80° C. is employed. The sterilization of blood serum is usually carried out in this manner, as it enables one to secure a medium which is semitransparent. When there are large numbers of spore-producing organisms present in the medium, it is sometimes impossible to sterilize by this means.

Sterilization by Filtration. — Sterilization may be effected by the filtration of liquids or gases through materials that will retain microörganisms.

Filtration of Gases by Cotton. — It is customary to plug with cotton the flasks, test tubes, etc., in which bacteria and other microörganisms are grown. In the discussion of spontaneous generation it was noted that air passed through cotton is robbed of its dust and microörganisms. It is therefore possible to sterilize media in tubes or flasks that have been plugged in this manner, and,

FIG. 79. — Various types of porcelain filters for sterilization of liquids by filtration.

if properly done, no organisms will develop, although the cotton will allow more or less interchange of gases between the inside and the outside. If, however, the cotton plug has become moist or if the medium is stored in a place that is too damp, molds and sometimes bacteria may grow down through the plug and appear on the inside, then, of course, contaminating the contained medium. When properly prepared and cared for, however, cotton plugs are efficient in the sterilization of the gases which enter the flask.

Filtration by Porcelain Filters. — Many filters constructed of unglazed porcelain, which will not permit the passage of micro-

organisms through them, have been placed upon the market. These are prepared in many sizes and shapes, the most commonly used being those that go by the name of the Berkefeld, the Pasteur, and the Chamberland filters. It is, of course, necessary that the vessels used and the filters themselves be sterilized before any attempt is made to remove bacteria by means of filtration. Filtration is particularly useful as a means of sterilization when we are dealing with substances like blood serum which are very easily changed by heat, particularly sera containing antitoxins, and all culture media containing toxins. These filters have been advocated as being useful in the purification of drinking water. In some cases they are screwed directly on the tap. It is found that in most cases the bacteria do penetrate these filters in the course of time, and such filters therefore must be sterilized at short intervals if they are to remain efficient. It is probably something other than mere size of the pores of the filter that determines its efficiency, because, as we have seen, bacteria in the course of time can grow through a filter which would ordinarily remove them from a liquid.

Sterilization by Light. — It will be noted later that certain rays of light, the blue, violet, and ultra-violet in particular, are destructive to living cells. Advantage has been taken of this in the construction of certain types of electric lights for the destruction of bacteria in water. A Cooper-Hewitt mercury vapor lamp with a quartz tube gives out a large proportion of these destructive ultra-violet rays, and has been put into practical use for sterilization of water in large quantities.

CHAPTER XI

USES AND PREPARATION OF CULTURE MEDIA

ANY nutrient material prepared in the laboratory for the growth and cultivation of microörganisms is termed a *culture medium.* Various kinds of culture media are necessary both to make it possible to keep organisms alive and growing, and to assist in the differentiation of species. Different media allow the organisms to exhibit their characteristic physiological activities as well as their morphology. Culture media are rendered particularly important because of the impracticability of differentiating closely related species by means of microscopic examination alone.

Characteristics of Nutrient Media. — A nutrient medium to be used for the growth of microörganisms must possess the following characteristics: first, it must contain nutrients suitable for the organism that it is desired to grow and in amounts suitable for its growth; second, it must possess a suitable reaction, and third, it must in most cases be rendered sterile.

1. *Nutrients used in Media.* — The particular food materials that must be present in a nutrient medium are determined by the species of organism to be grown. Some require no organic matter of any kind, others require such specialized types as blood serum. Between these extremes are to be found those having less specialized requirements, including the greater number of organisms that produce fermentation and decay, and most of those that cause disease.

2. *Reaction of Medium.* — Microörganisms will not grow in a medium that is too acid or too alkaline. It is therefore necessary to adjust the reaction of most media before use. Species of microorganisms differ in their optimum reaction. In making comparative tests it is of the greatest importance that the reaction of the

medium should be uniform. Methods of adjusting the reaction are given below.

3. *Sterility of Medium.* — After the preparation of a medium and before it can be used for culture it must be completely freed from living organisms of all kinds. This process, termed sterilization, has already been discussed.

Adjustment of the Reaction of the Medium. — The reaction of a medium, that is, its relative acidity or alkalinity, may be designated in one of two ways: first, by the amount of normal acid or normal alkali[1] required to bring one hundred cubic centimeters of the medium to the neutral point of some particular indicator, or second, by the designation of the true hydrogen ion concentration or, conversely, of the true hydroxyl ion concentration of the medium. The first method was formerly the more commonly used, the second method is in most instances preferable and is rapidly replacing the former. It is this which will be discussed.

Bacteria are usually quite sensitive to the presence of an excess of acid or alkali. Some grow best in a medium which is strictly neutral, others prefer one which is somewhat on the acid side of neutrality, still others one on the alkaline line of neutrality. Careful adjustment of reactions is therefore necessary in many cases. Some organisms, for example, will not grow unless the medium has almost exactly the same reaction as that of the blood. Other organisms, as the yeasts, grow better in a medium which is somewhat acid.

A concept which relates reaction to our senses, and is helpful in understanding this matter, is that of sourness. One may determine the amount of acid in a solution by titrating against a suitable

[1] A normal solution of an acid is one that contains "the hydrogen equivalent in grams per liter," or practically one gram of acid (replaceable) hydrogen per liter of solution (accurately 1.008 grams per liter). To prepare a normal solution, the molecular weight of the acid is calculated, and, if the acid is monobasic, this amount in grams is made up to one liter of solution. If more than one acid hydrogen atom is present in the molecule, the molecular weight is divided by the number of such atoms. For example, 60 grams of acetic acid (CH_3COOH, molecular weight 60) made to a liter of solution with distilled water is a normal solution. In normal sulphuric acid (H_2SO_4, molecular weight 98) 49 grams are present per liter. Normal alkalies are those that neutralize exactly equal volumes of normal acids. In descriptions of culture media acidities are usually indicated by the plus (+) sign, alkalinities by the negative (−) sign. A reaction of + 1.0 means that in 100 cc. of the medium, there is an equivalent of 1 cc. of normal acid. In the determination of the reaction phenolphthalein is used generally as an indicator.

standard solution. But this does not measure the true acidity. This latter is due to the presence of hydrogen ions which have been dissociated from the acid. Alkalinity, on the other hand, is due to the presence of dissociated hydroxyl ions. The true acidity is found to be determined not only by the amount of acid but by the kind of acid as well. Hydrogen ions are dissociated in relatively large amounts by so-called strong acids, and in small amounts by weak acids. Equal concentrations of strong and weak acids show therefore marked differences in true acidity, *i.e.*, in hydrogen ion concentrations. In addition, the hydrogen ion concentration of a solution may be modified by substances which modify the degree of dissociation of the acid; these are called buffers, and will be discussed below. It is evident therefore that the true acidity (sourness) of a solution depends on at least three important factors:

1. The amount of acid present.
2. The kind of acid present.
3. The buffer present.

Several scales have been developed which are useful in designating the true reaction of any solution. One of these scales is that which is used to designate the hydrogen ion concentration of a solution. Its development and use may be understood from the following discussion.

Pure distilled water is neutral, that is, when a molecule of water dissociates, it breaks up into equal numbers of hydrogen and hydroxyl ions. The physical chemist has been able to prove, furthermore, that pure water (and therefore any neutral solution in water) contains approximately one ten-millionth of a gram (10^{-7} grams) of hydrogen ions to the liter. The concentration may be still more conveniently expressed in terms of normality of hydrogen ions. A normal solution of hydrogen ions is one which contains one gram of hydrogen ions per liter. A neutral solution, therefore, is one which has a concentration of hydrogen ions of 10^{-7} normal. Since in a neutral solution there is the same number of hydroxyl ions as of hydrogen ions, the hydroxyl ion concentration must also be 10^{-7} normal.

The chemist has also shown that in any solution the *product of*

the normality of hydrogen ions by the normality of hydroxyl ions is a constant number (the so-called *dissociation constant*). The numerical value of this number may be determined by multiplying 10^{-7} (normality of hydrogen ions in a neutral solution) by 10^{-7} (normality of hydroxyl ions in a neutral solution). This product is 10^{-14}. In other words, the product of the normality of the hydrogen ion concentration of a solution by the normality of the hydroxyl ion concentration must always be approximately 10^{-14}. If we know either the hydroxyl or hydrogen ion concentration, we can at once determine the concentration of the other. For example, if we are dealing with a solution having an hydrogen ion concentration of 10^{-4} normal, its hydroxyl ion concentration must be 10^{-10} normal. A scale to designate the acidity of any solution in terms of its hydrogen ion concentration may be arranged as follows:

10^{0} 10^{-1} 10^{-2} 10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7} 10^{-8} 10^{-9} 10^{-10} 10^{-11} 10^{-12} 10^{-13} 10^{-14}

On this scale it will be noted that the larger the numerical value of the exponent, the smaller the hydrogen ion concentration. It will be recalled that values to the right of 10^{-7} represent increasing values of alkalinity or decreasing hydrogen ion concentration and numbers to the left represent increasing acidity. Inasmuch as this method of statement is somewhat cumbersome, it has been suggested by Sörenson that the exponents be used to indicate the scale, dropping the negative signs. This gives the scale:

0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14

Each of these numbers is termed the pH [1] of a solution. This abbreviation comes from the fact that an electrometric method of measuring hydrogen ion concentration is frequently used in which the pH is determined from the electrical potential produced by the hydrogen ions, *i.e.*, the p stands for "potential."

A solution having a pH of 0, for example, would have a normality of hydrogen ion concentration of 10^{0} or 1, one having a pH value of 7 would have a hydrogen ion concentration of 10^{-7} normal, that is, it would be neutral. It is evident, therefore, that the smaller

[1] Technically the pH value is the logarithm of the reciprocal hydrogen ion concentration. However, this is simply another way of saying that the exponents of the hydrogen ion concentration as noted in the scale above are used, changing the negative signs to positive.

the number on this scale the higher the hydrogen ion concentration, that is, the greater the actual acidity, and the larger the number the greater the actual alkalinity.

One characteristic of this scale must be kept in mind. Each number represents a hydrogen ion concentration ten times that of the number to the right. For example, the hydrogen ion concentration of a solution in which pH = 6 is ten times that of one with pH = 7.

Indicators are chemical substances which are one color in a certain range of pH values or hydrogen ion concentrations, and another color in other ranges. The indicator most commonly used is litmus, which has a lilac color at true neutrality, that is, at a pH value of 7. At a pH value of 8, that is, in a more alkaline solution, it is blue. At a pH value of 5, that is, in a stronger concentration of hydrogen ions, it is red. Other indicators change color at other pH values. For example, phenolphthalein is colorless in all hydrogen ion concentrations having a pH value less than 8.2, in more alkaline solutions it is red. Many other indicators are known which change color at other points in the hydrogen ion or pH scale. This change of color is not instantaneous. In adding alkali, for example, to an acid solution containing litmus there is not a transformation of red into blue at a particular or definite pH. The color changes gradually with the addition of a base, that is, there is a *range* of pH values over which the color change occurs. By the use of standards whose hydrogen ion concentration is known it is possible to determine approximately the hydrogen ion concentration of any material which it is desired to test by the use of the intensity of color of appropriate indicators. This will be discussed in Chapter XIV.

Most bacteria grow in a hydrogen ion concentration of 10^{-7} to 10^{-8}, that is, in solutions having a pH value between 7 and 8. The pH value of blood is usually about 7.35. This indicates therefore the hydrogen ion concentration most useful in cultivating many species of pathogenic bacteria. Since most media are on the acid side of true neutrality, it is necessary to add alkali, usually potassium or sodium hydroxide, until test shows that the hydrogen ion concentration has become satisfactory. The actual adjustment of

the reaction is accomplished by determining how much of a standard base (as $\frac{N}{20}$ NaOH) is required to bring a measured portion of the medium to the desired pH as shown by the color of a suitable indicator. From this the amount of standard alkali required to adjust the reaction of the entire amount may be calculated.

The hydrogen ion concentration of a medium or solution depends not only upon the actual concentration of the acid itself but upon the concentration of substances which are termed *buffers*. A buffer is any substance in a solution which tends to prevent marked changes in hydrogen ion concentration upon the additions of alkalies or acids. Certain salts, particularly the phosphates and carbonates and many organic substances, such as the amino acids and peptones, act in this manner. For example, the addition of a small amount of an acid to distilled water will give a marked change in the hydrogen ion concentration, but the same amount of acid added to solutions containing considerable amounts of buffers may result in very slight differences in hydrogen ion concentration. It is evident that inasmuch as microörganisms are affected far more by differences in hydrogen ion concentration than they are by the total amount of acid present, heavily buffered media are preferred for the growth of microörganisms.

When a medium has been finally adjusted in its reaction, that is, when the right amount of alkali or acid has been added to give the desired hydrogen ion concentration, it is frequently necessary to boil or heat it to precipitate out any materials not soluble in boiling water at the new hydrogen ion concentration. This is followed by filtration. If this step is omitted, the medium will ordinarily be cloudy or may yield a sediment.

In summary, it may be well again to emphasize that the true acidity of a solution is determined principally by three distinct factors, the amount of acid present, the kind of acid (weak or strong) present, and the extent of the buffering action of various substances present.

Principal Types of Media. — It is impracticable here to enter into a discussion of all the types of media that have been proposed and used for special purposes. Of these there are thousands.

There are a few, however, that have proved suitable to a wide range of organisms, and these may be considered briefly.

Media may be divided into two groups, *non-synthetic* and *synthetic*. A *non-synthetic medium* is one in which the exact chemical composition of each of the constituents is not certainly known; frequently even the exact percentage of these constituents is uncertain. Such media, for example, are potato and milk. In a synthetic medium on the other hand only pure chemicals of known composition are used. The use of a synthetic medium is distinctly advantageous when it is desired to determine certain physiological characteristics of organisms. A medium in some cases may be non-synthetic in part, but the addition of certain chemicals, as the sugars, may make possible the recognition of fermentative capacity of the organisms being studied. Media may also be divided into three classes on the basis of consistency: liquid media, liquefiable solid media, and non-liquefiable solid media.

The preparation of many media has been greatly simplified in recent years by the introduction of desiccated preparations. These when dissolved in water, tubed, and sterilized, are ready to use.

Non-synthetic Media

Liquid Media. — The most commonly used of the non-synthetic liquid media are *beef broth* or *bouillon*, *milk*, *blood serum*, and *beer-wort*, and their various combinations and modifications.

Nutrient Broth or Bouillon. — This is made in one of two ways: either directly from meat or from meat extract. In the preparation of the first type, 500 grams of lean minced beef is soaked in water (preferably distilled water) in the ice chest for twenty-four hours, squeezed through cheesecloth by means of a meat press, and the filtrate made up to one liter. To this is added 1 per cent (10 grams) of a standard peptone, and $\frac{1}{2}$ per cent (5 grams) of sodium chloride. Peptone is a mixture of albumoses and peptones derived from the digestion of protein by pepsin; it may be considered as partially digested meat. It differs from the protein from which it is derived by being soluble in boiling water. These materials are brought into solution, the reaction adjusted, boiled for five minutes, filtered, and sterilized.

Preparation of bouillon from meat extract is very similar except that of beef extract 3 grams in a liter of water is used in place of the beef infusion. Broth is frequently modified by the addition of sugars or glycerol.

Dunham's Solution. — This consists of 0.5 per cent sodium chloride and 1 per cent Witte's peptone in water. As will be noted later, it is commonly used in the determination of indol production by bacteria.

Milk. — Milk is a suitable medium for the growth of a great number of organisms, as it contains carbohydrates, fats, and proteins, and when fresh, possesses a suitable reaction. Separated milk should be used, certified milk if it can be procured. If separated milk cannot be secured, the milk may be pipetted from below the cream layer of a flask kept in the refrigerator for twenty-four hours. It is best sterilized in the Arnold, but where great accuracy is not required, it may be sterilized in the autoclave. Milk is commonly tinted blue by the addition of sterile litmus to act as an indicator of the development of acid.

Blood Serum. — Sterile liquid blood serum, alone or mixed with broth, is sometimes used as a medium. It is difficult to sterilize such serum after removal from the body without causing it to coagulate; hence, the necessary precautions to insure sterility and prevent contamination are taken at the time the blood is drawn. It is allowed to stand in a sterile vessel until it clots, then the serum is pipetted off.

Beerwort. — Unhopped beerwort is a very useful medium for the cultivation of yeasts, molds, and some bacteria. It is prepared by soaking malted grain, usually barley, in water. The starch of the grain is changed to sugar by the diastase present, and this malt sugar (maltose), together with certain of the protein constituents of the grain, passes into solution. It should be boiled and filtered to remove all substances coagulated by heat before being placed in test tubes and sterilized. The desiccated malt extract may also be used for this purpose.

Liquefiable Solid Media. — Liquid media may be made solid by the addition of gelatin or agar-agar. Gelatin is prepared by boiling bones, joints, and tendons. It has the property of solidify-

ing or gelatinizing when cool and of being liquid when warm. Gelatin alone, dissolved in water, is a medium upon which many organisms can grow. The addition of gelatin to bouillon or beerwort transforms them into media which are liquid when warm and solid when cold. Chemically, gelatin is a protein. Agar-agar is a gumlike material obtained from certain seaweeds, particularly forms native to the Asiatic coast of the Pacific Ocean. It has long been used by the Chinese in the thickening of soups. Like gelatin it may be dissolved in hot water, and when cool, will cause it to gelatinize. It differs, however, from gelatin in that it is not nitrogenous but is related to the vegetable gums. Very few organisms indeed are known which are capable of liquefying this substance or of utilizing it as food; therefore, when it is used as a means of rendering a medium solid, it does not ordinarily add any nutrient, and any growth of the organisms which takes place is due to the other constituents of the medium. Gelatin, on the other hand, may be digested and liquefied by many species of bacteria and molds. Nutrient agar does not liquefy until it has been heated to a temperature only a few degrees below the boiling point of water, but may be cooled down to a temperature of 38° to 40° C. before solidifying. Gelatin, on the other hand, liquefies even at blood heat, and when used as a culture medium during hot weather, it is necessary to keep it in a suitable thermostat.

Nutrient or Bouillon Gelatin. — Nutrient gelatin is prepared by the addition of 10 to 15 per cent of the best gold-label gelatin to bouillon. It is customary to cool the medium down to below 60° C. after the gelatin has gone into solution and add the white of an egg. The medium is then heated until the egg white has completely coagulated. This carries down many of the finer particles that have been in suspension, and renders the medium perfectly clear. Gelatin may be sterilized in the autoclave, provided a pressure of not more than ten pounds for fifteen minutes is used. The application of too much heat, or heat applied for too long a time, will destroy the power of gelatin to solidify when cooled. Gelatin itself is usually somewhat acid; it is therefore necessary to neutralize after the addition of the gelatin to the medium.

Nutrient or Bouillon Agar. — Agar (1.5 per cent) is added to nutrient bouillon and heated until it has passed completely into solution. Frequently laboratory workers prepare a bouillon or broth of double strength and dissolve the agar needed in an equal amount of water by means of the autoclave, and then mix the two solutions. Agar is neutral in its reaction; hence it will not change the reaction of the medium to which it is added. It is not injured by heating; the sterilization of media containing agar may therefore be easily effected by the use of the autoclave. Nutrient agar is one of the most common media employed in the laboratory.

Sugar Gelatin and Sugar Agar. — Gelatin and agar may be modified by the addition of various sugars, or in some cases by the addition of both sugar and litmus.

Glycerol Gelatin and Glycerol Agar. — The addition of glycerol to the gelatin or agar renders them much more suitable for the cultivation of a few pathogenic bacteria.

Beerwort Gelatin and Beerwort Agar. — Beerwort may be transformed into a liquefiable solid medium by the addition of either gelatin or agar, as has been described. These media are particularly valuable for the cultivation of yeasts and molds. It is not customary to neutralize the beerwort, as both yeasts and molds flourish better upon a medium which is somewhat acid in reaction.

Non-liquefiable Solid Media. — The media belonging to this general type most in present use are potato, coagulated blood serum, and coagulated egg. In addition certain other materials are sometimes used, such as carrots, peas, beans, etc.

Potato. — Sterilized potato as a medium was one of the first introduced into the laboratory. It is customary to use cylinders cut from the potatoes by means of an apple corer or special potato borer. These cylinders are then divided longitudinally by a diagonal cut and placed in running water for a few hours. This treatment prevents them from turning black when sterilized later. They are then placed with the slope up in special potato tubes, such as illustrated in Fig. 80, or in large test tubes having a small mass of cotton in the bottom. The bulb of the special potato tube should be entirely filled with water, or in the other tubes the cotton saturated. This prevents the potatoes from drying out too

rapidly. Sterilization is effected in the autoclave at fifteen pounds' pressure for fifteen minutes. Potatoes are more difficult to sterilize than some other media because they ordinarily are covered with spore-producing bacteria of a particularly resistant type.

Carrots, Beans, etc. — Carrots are sometimes prepared in the same manner as outlined above for potatoes. The sterilized pods of green string beans are likewise useful for the cultivation of certain molds. Starch paste, moistened corn meal, bread crumbs, etc., are sometimes utilized for the study of special forms.

Blood Serum. — Blood serum, either alone or mixed with bouillon or glycerol, is coagulated in a slanting position in test tubes by means of heat. Such media are particularly useful in the cultivation of certain pathogenic bacteria. A satisfactory substitute for this may be prepared by mixing the white and yolk of an egg and sterilizing in the same manner.

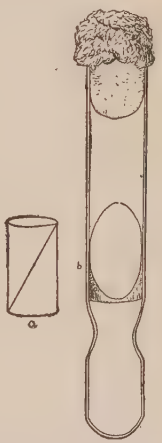


FIG. 80. — Potato tube and preparation of potato as a medium. *a*, cylinder of potato showing diagonal longitudinal cut; *b*, potato tube with slanted potato surface.

SYNTHETIC MEDIA

It is sometimes necessary to use a medium whose exact chemical constitution is known in order that the factors determining the growth of the microörganisms may be determined and the products of their activity studied by analytical methods. Many kinds of synthetic media have been proposed for definite specific purposes. It is always necessary in such a medium to see that certain salts are present, usually about 0.5 per cent of sodium chloride with traces of calcium, magnesium, potassium, and phosphorus salts as well. Some of the bacteria develop only upon media having some protein material present. For these, of course, it is necessary to use non-synthetic media. The majority will grow, provided there are suitable sugars or glycerol in addition to salts such as those of ammonia or of nitric acid, capable of furnishing nitrogen. Synthetic media may be solidified by the addition of agar.

CHAPTER XII

PURE CULTURE METHODS IN BACTERIOLOGY

Pure and Mixed Cultures. — Any growth of organisms on laboratory media is termed a *culture*. Such a growth that has originated from a single organism or spore is called a *colony*. A culture in which only one species of organism is present is said to be *pure;* one in which several are present is *mixed*.

In general it is necessary that an organism be in pure culture before it can be studied, or at least before its distinctive physiological characteristics can be determined. It is unusual to find organisms in pure culture in nature; many species occur together in most situations. Methods for resolving such natural mixtures are therefore of fundamental importance. Each organism must be separated from all others and grown in pure culture. Many methods have been devised, the most important of which will be briefly noted.

Methods of Transfer. — Microörganisms, particularly bacteria, are transferred from one medium to another for cultivation, or to a microscopic slide for examination, by means of one of two instruments, the glass pipette and the platinum needle or loop. The pipette (sterilized in the hot-air oven) is used with liquids when it is necessary to transfer a definite quantity. The platinum needle is prepared by fusing a piece of platinum wire two to three inches long into the end of a glass rod or other suitable handle. It is sterilized by heating to a glow in the flame of the bunsen. After cooling, it may be touched to a bacterial culture. A sufficient number of bacteria will adhere to enable one to make a planting upon another medium. For transferring small quantities of liquids a loop is made in the end of the wire (Fig. 81).

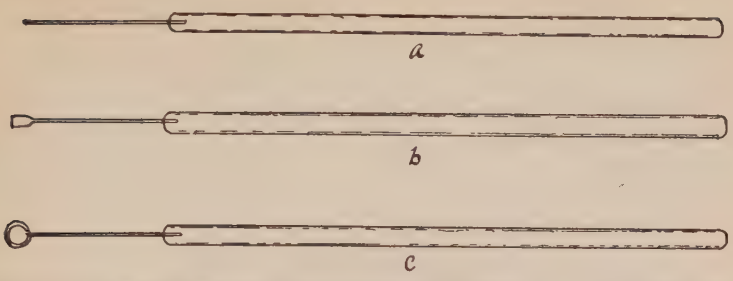


FIG. 81. — Platinum wires and loops used in the laboratory. *a*, straight wire, *b*, spatulate wire; *c*, platinum loop or oese.

METHODS OF SECURING PURE CULTURES

Direct Transfer. — In diseases of man and animals pure cultures may often be made directly from the part of the body infected, as from the blood or the spleen in typhoid fever. Bacterial and yeast cells are so small that it is relatively difficult to pick up a single individual and separate it from all other organisms that may be present. In recent years an apparatus has been developed by means of which it is possible to pick up a single cell, the operation being watched by means of the microscope. The method is tedious and difficult and, except for some special purposes, not adapted to routine laboratory work. It has proved very valuable, however, for certain purposes. Molds, on the other hand, frequently may be isolated at once in pure culture by touching a sterile needle to the spore masses. Usually these masses are free from other organisms. This is particularly true of those forms that send up erect conidiophores or sporangiophores.

Streaking. — A mixture of various organisms, if smeared over the surface of a solid culture medium in successive streaks, will not be uniformly distributed. The various bacteria will grow in a mass where most thickly inoculated; they will develop distinct colonies where fewer are planted. Microscopic examination will often show some of these to be made up of a single species of organism (Fig. 82). Transfers may then be made from such colonies to other media and a pure culture thus secured.

Dilution. — This method was the first employed in the separation of mixed cultures, particularly in the early study of yeasts. De-

creasing amounts of the original inoculating material are added to a series of flasks or tubes containing a liquid medium. This is usually accomplished by adding a measured amount, 1 cc. for example, to a flask of nutrient solution, the same amount from this flask is inoculated into a second, from this to a third, and so on. It is evident that, if a sufficient number of dilutions is made, some of the higher dilutions will contain no organisms and in consequence will show no growth. Some of those showing growth will, upon microscopic examination, be found to contain but a single species; they will be, in other words, pure cultures. It is evident that such a method must be cumbersome and poorly adapted to laboratory work for most cases. It has been almost wholly superseded by better and more expeditious methods.

FIG. 82. — The isolated colonies of a streptococcus secured by streaking on the surface of an agar medium. (After Thompson.)

Plate Cultures. — The discovery and introduction of liquefiable solid media, such as gelatin and agar, greatly simplified the isolation of pure cultures. Varying amounts of the material from which isolations are to be made are introduced into tubes of suitable nutrient medium containing gelatin or agar. This medium has been first liquefied by heat and then cooled to a temperature of about 42° C. This temperature will keep the medium liquid and is not high enough to injure the microörganisms introduced. The inoculated medium is then poured into a *petri dish*. This is a shallow, circular, flat glass dish with a glass cover. The medium is uniformly distributed over the bottom of the dish and the latter

then placed upon a cool surface to cause the medium to solidify quickly. The petri dish is often called a *plate*, and this process is termed *plating* or *plating out*. It is evident that the microörganisms present are separated from each other. The solidification of the medium prevents them from moving about. They are surrounded with suitable nutrients and begin to grow. In the

FIG. 83. — Isolation of pure cultures by plating. An agar plate showing the isolated colonies from which pure cultures may be secured.

course of a few hours or days each organism will grow: if yeast or bacteria, the cells will multiply or, if mold, increase in size until the mass becomes visible to the unaided eye as a colony (Fig. 83). In most instances each colony originates from a single organism, and is therefore a pure culture from which transfers may be made directly to suitable media. The simplicity of this method has made it the one most commonly used in the laboratory.

Selective Media, Chemicals, and Heat. — A medium suited to the growth of one species of organism may be wholly unsuited to another. The addition of certain chemicals, such as a weak solution of carbolic acid, may effectually prevent the development of any other than a single form. The use of bile will inhibit the growth of most bacteria other than those found in the intestinal tract. This inhibition method is used most extensively in the isolation of pathogenic and intestinal bacteria from polluted waters.

A spore-bearing organism frequently may be isolated from a mixture containing non-sporulating forms by heating to a temperature sufficient to kill everything but the spores. Usually 80° C. for thirty minutes is sufficient. The spores begin to develop as a pure culture when the medium is cooled.

Animal Inoculation. — Some bacteria capable of producing disease in man or animals do not grow rapidly or readily on culture media, and not at all upon liquefiable media. When these are present in impure cultures, as, for example, the tubercle bacilli in the sputum of a consumptive, it is sometimes necessary to inject the mixture into a susceptible animal, such as a mouse, rabbit, or guinea pig. The body destroys all of the bacteria except the one capable of causing disease. Later the animal may be killed and the organism desired be isolated in pure culture from one of the glands or internal organs.

CHAPTER XIII

STUDY OF GROWTH CHARACTERS IN PURE CULTURES

The growth characteristics of bacteria in cultures are often useful in assisting in the differentiation of species. The same is true with yeasts, and to a less degree with the molds. In the latter, the classification is often based wholly on morphology.

The Society of American Bacteriologists, through a committee, has evolved and adopted a very satisfactory outline for such records. Copies of this chart are kept in most laboratories, and should be studied in connection with the contents of this chapter. This chart must be modified in some instances to fit special cases, but is broad enough to cover most forms. It will serve as a basis for the following discussion.

Cultural Characters of Bacteria

The culture to be studied may be a colony on an agar or gelatin plate, a slant or streak culture on some solid medium, a stab culture in gelatin or agar, or a culture in a liquid medium, such as broth or milk.

Colonies on Agar and Gelatin Plates. — The appearance of colonies of the same organism may differ as a result of variations in the composition of the medium. A medium with a moist surface, for example, will give a different type of colony from one whose surface is drier. It is important, therefore, that conditions, both as to composition of the medium and the environment, shall be kept as uniform as possible. It is evident that slight differences in colony form cannot be made a basis for species determination.

Examinations of the colony are to be made both by means of the unaided eye (macroscopic) and with low magnification (microscopic), using a hand lens or the low power of the microscope.

Both colonies at the surface and those entirely below the surface (deep colonies) should be examined. The macroscopic examination includes a determination of the size, form, surface elevation, margin, and topography.

The **size** of the colony is to be expressed in millimeters. This is, of course, a somewhat variable quantity, depending upon the age of the culture.

The **form** of the colony may be described briefly. The following terms are useful, but are by no means the only ones that may be

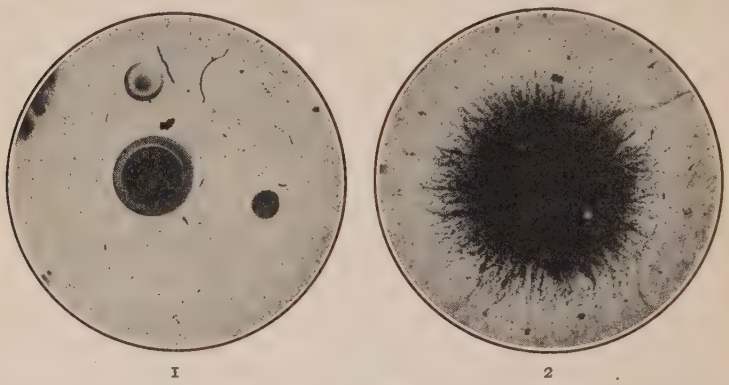


FIG. 84. — Bacterial colonies. 1, circular colony with entire margin, umbonate in cross section as the center is thicker than the edge; 2, myceloid colony, the filaments or chains of bacteria radiating from the center.

used for this purpose. A *punctiform* colony is one that is just visible to the naked eye as a minute dot. Colonies are frequently *circular*, *oval*, or *spindle shaped* (*fusiform*). An *amœboid* colony is one that is very irregular in shape.

The **structure** of the colony can sometimes be determined by the naked eye; usually a lens is necessary. A *myceloid* colony is one which shows radiating filaments resembling the mycelium of a mold. A *filamentous* colony differs from the preceding in that the threads are irregularly intertwined and not radiating from the center. A *rhizoid* colony is one which shows an irregular, rootlike system of branching.

The term **surface elevation** indicates the relative thickness of the mass of organisms. A thin, spreading colony is said to be *flat*.

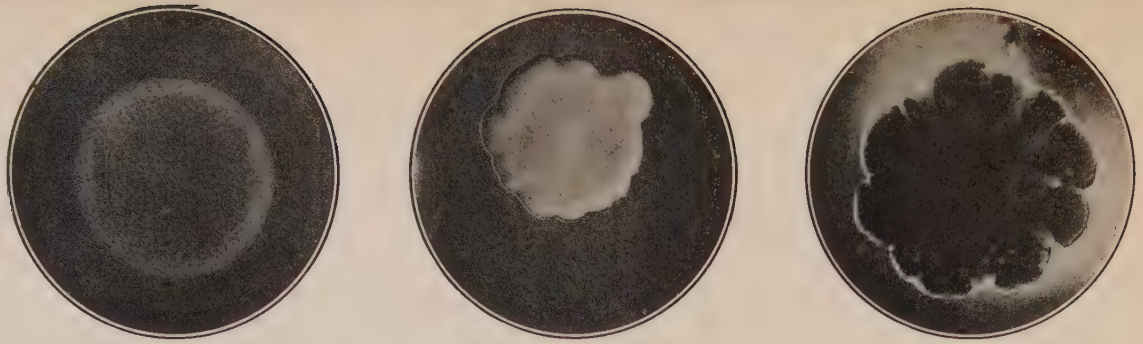


FIG. 85. — Types of bacterial colonies. 1, circular colony, with entire margin smooth, structure homogeneous; 2, colony circular, margin lobate, smooth, homogeneous; 3, circular colony, margin auriculate, smooth. (Photomicrographs, × 10.)

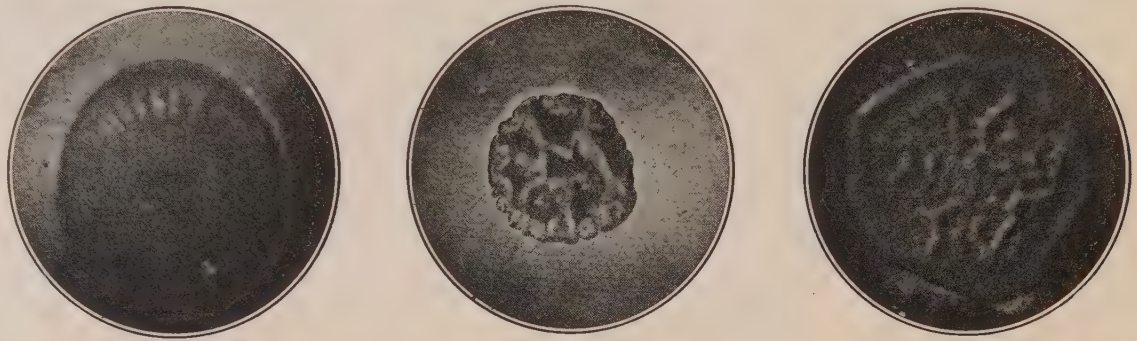


FIG. 86. — Types of bacterial colonies. 1, circular colony with radial wrinkling of the surface, umbilicate in cross section; 2, colony with thick irregular wrinkling of the entire surface; 3, colony with wrinkling not extending to the margin. Enlarged.

If scarcely visible, as a very delicate film over the surface, it is said to be *effuse*. A *raised* colony is one which is uniformly thickened and with a well-defined margin. A *convex* colony is one which is somewhat thicker in the middle than at the edges. A *pulvinate* colony is one which is decidedly convex. A *capitate* colony is one that is hemispherical. A colony having a knob or elevation near its center is said to be *umbonate;* one that has a depression at the center is termed *umbilicate*. If the colony studied is upon gelatin, it may liquefy the medium. The **margin** of a colony, when viewed under the low power of the microscope, may be entire, or without irregularities; *undulate*, or wavy; *lobate*, with rounded lobes; *lacerate*, as if torn or shredded; *fimbriate*, with a fringe; *filamentous*, consisting of filaments like delicate hyphæ; or *curled*, like a cluster of hairs.

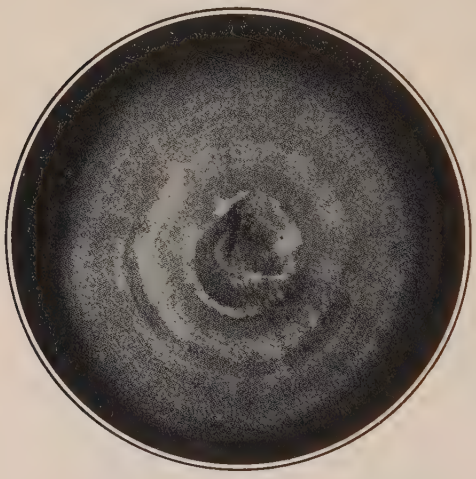


FIG. 87. — Colony showing concentric rings. (× 40.)

The **topography** of the surface of a colony may be *smooth*, *rough*, *ringed*, *radiate*, or *striate*.

Slant or Streak Cultures on Solid Media. — A slant, slope, or streak culture of an organism is prepared by drawing an inoculated needle in a straight line over the surface of a medium. This medium may be one rendered solid with agar or gelatin; it may be coagulated blood serum or egg; or the cut surface of a potato, starch paste, etc. It is customary to note in these cultures the amount and form of growth (Fig. 94), its elevation, luster, topography, color, odor, consistency, and any changes produced in the medium.

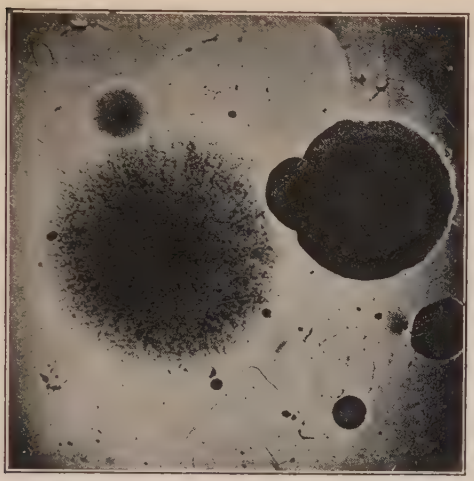


FIG. 88. — Bacterial colonies on agar plate. Large colony to left is floccose, large colony to right shows concentric markings. (× 20.)

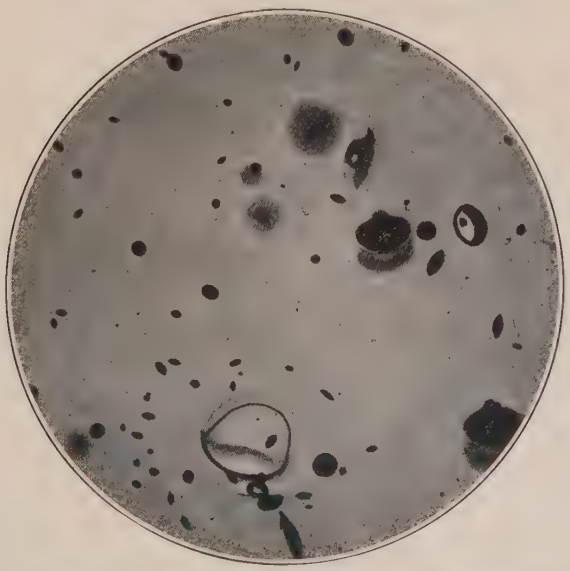


FIG. 89. — Portion of a plate culture showing a considerable number of deep spherical and lens-shaped colonies. (× 10.)

The **amount of growth** may be described as *invisible*, *scanty*, *moderate*, or *abundant*. These terms are only relative and some experience is necessary for their differentiation.

The form of growth may be *filiform*, extending but little to either side of the line of inoculation; *echinulate*, somewhat wavy or roughened on the margins; *beaded*, the organism growing as more or less minute separate colonies; *effuse* or *spreading*, as a thin indefinite layer over a large portion of the surface; *plumose*, or feathery; *arborescent*, giving off shoots causing the culture to resemble a fir tree; or *rhizoid*, with rootlike branches (Fig. 94).

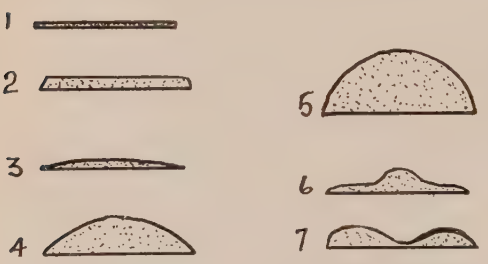


FIG. 90. — Cross sections of various types of colonies. 1, flat; 2, raised; 3, convex; 4, pulvinate; 5, capitate; 6, umbonate, 7, umbilicate.

The terms describing **elevation of growth** are much the same as those for colonies; those most commonly used are *flat*, *effuse*, *raised*, and *convex*.

The **luster** of the culture is determined by the use of reflected light. It may be *glistening*, *dull*, or *cretaceous* (chalky).

The **topography** of the surface may be *smooth*, *contoured* (with an irregular and smoothly wavy or undulating surface), *rugose* (wrinkled), or *verrucose* (covered with warts).

The **optical characters** are determined by the use of transmitted light. The culture may not transmit light (*opaque*) or it may be *translucent* or *transparent*.

The **chromogenesis** or color production of an organism should be noted. The pigment may remain wholly within the mass of growth, or it may diffuse through the medium. The solubility of the pigment may also be determined in water, ether, alcohol, and chloroform.

The odor may be recorded as *absent*, *slight*, or *decided*. If possible, resemblance to other odors should be noted.

The **consistency** of the culture is most readily determined by touching with a sterile platinum wire. It may be *slimy, butyrous* (resembling butter), *viscid, waxy, membranous* (difficult to detach from the medium, usually entire colony coming off together), or *brittle*.

The **changes in the medium** may consist of liquefaction with gelatin, blood serum, and similar media, or of a change in color. If the latter occurs, the exact change should be recorded.

Stab or Stick Cultures in Solid Media. — These are prepared in media solidified by agar or gelatin, by inserting a straight inoculated platinum needle for some distance. The growth on the surface of the medium may be described in the same manner as for colonies. The principal points to note in these cultures are: growth along the line of puncture, and changes occurring in the medium (Fig. 96).

The **growth along**

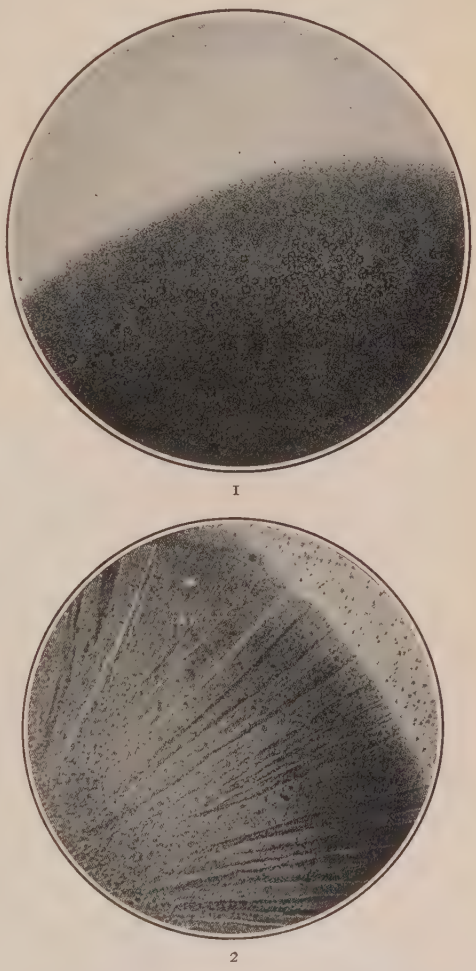


FIG. 91. — Colony margins and structure. 1, entire margin, finely granular at margin, interior somewhat grumose; 2, margin entire, with distinct radial markings or striæ.

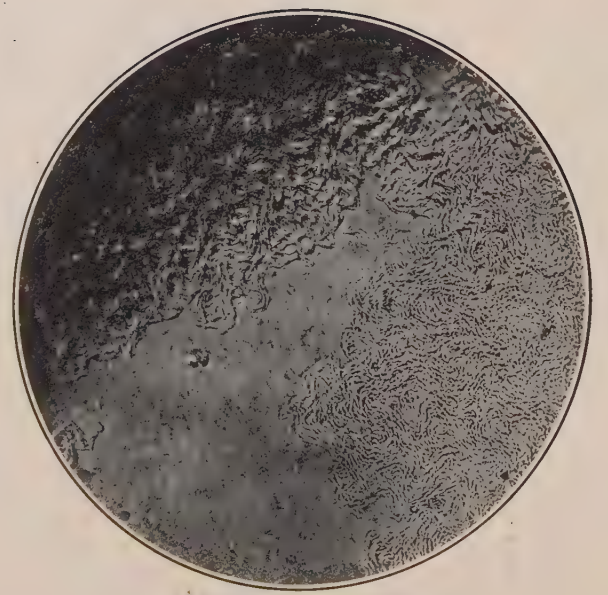


FIG. 92. — Colony margin and structure. Curled margin, interior slightly granular and wrinkled.

FIG. 93. — Margin of a very irregular colony (lacerate). (× 100.)

the line of puncture may be distributed *uniformly;* it may be *best at the top or best at the bottom.* It may be *filiform* or uniform; beaded, consisting of more or less distinctly separated colonies; *papillate*, covered with small papillæ; *villous*, covered with straight hairlike projections; *plumose*, like a feather; or *arborescent*, like a tree with a main trunk and branches (Fig. 95).

The principal **change in medium** to be noted is liquefaction. The liquefied portion may be *crateriform*, or saucer shaped; *napi-*

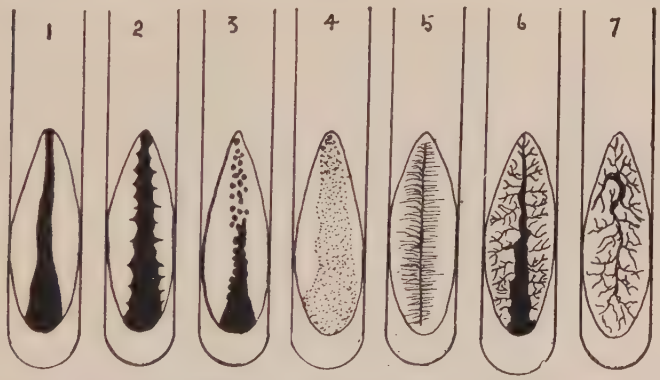


FIG. 94. — Forms of growth on streak cultures. 1, filiform; 2, echinulate; 3, beaded; 4, effuse; 5, plumose; 6, arborescent; 7, rhizoid.

form, like a turnip; *infundibuliform*, funnel shaped; *saccate*, sack shaped; or *stratiform*, as a horizontal stratum. Changes in the color of the medium should also be noted.

Cultures in Liquid Media. — These may be made in some transparent medium as broth, or in one of the synthetic media, or milk. In the clear media it is necessary to observe the surface growth, clouding, sediment, and odor. In milk (usually litmus milk) the coagulation, digestion of casein, reaction, and consistency should be noted.

Some of these latter changes will be discussed in greater detail under the heading of physiological characters.

Surface growth in broth may occur as a *ring* where the surface of the medium meets the walls, or as a more or less decided membrane or *pellicle*. It may remain persistently at the surface or it

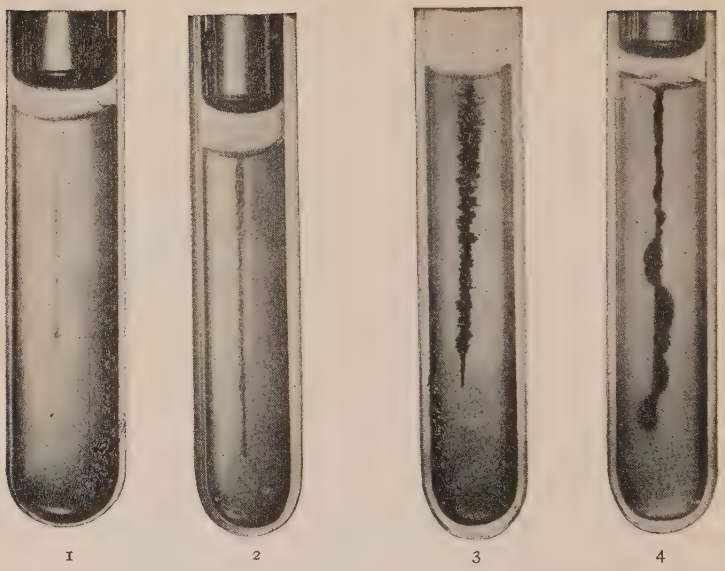


FIG. 95. — Types of growth in stab cultures. 1, scanty; 2, filiform; 3, villous; 4, winged.

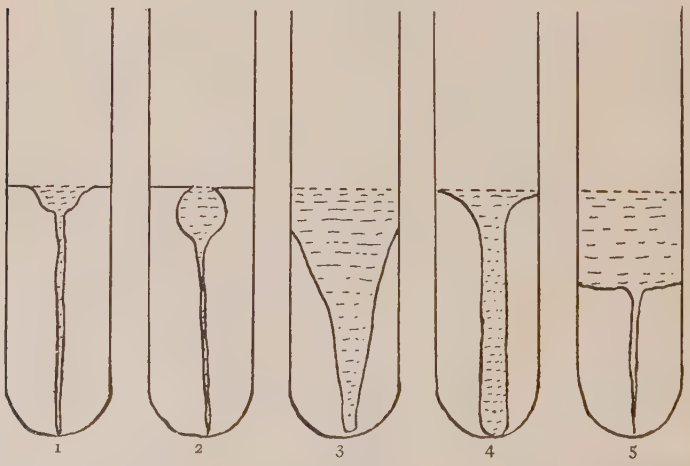


FIG. 96. — Types of liquefaction in gelatin stab cultures. 1, crateriform 2, napiform; 3, infundibuli; 4, saccate; 5, stratiform.

may be easily shaken down to the bottom. It may be *friable*, *firm*, *membranous*, or even *leathery*. With many organisms no surface growth appears.

The clouding of broth may be *absent*, *slight*, *moderate*, or *strong*.

The **sediment** may be *absent*, *scanty*, or *abundant* in amount, and in consistency *compact*, *flocculent*, *granular*, or *viscid*.

FIG. 97. — Margin of a colony of yeast. (× 100.)

Milk may be curdled promptly, after the lapse of considerable time, or not at all; and the curd formed may or may not shrink with expulsion of the whey, and it may or may not be digested. The curd may be formed as a result of the development of acid or of a rennet-like enzyme. Most organisms that digest the casein actively produce coagulation by the second method; the acid-forming bacteria in most cases do not digest the casein. The reaction may remain unchanged, or it may become acid or alkaline, or first acid and then alkaline. The consistency of the milk may remain unchanged, or it may become slimy or viscid.

Yeasts may be studied in exactly the same manner as bacteria

with reference to their appearance when grown in cultures. The same descriptive terms may be used. The types of media best adapted for their growth, however, are not the same as those most favorable for bacteria. Sugar broths, synthetic media, wort, and must with or without the addition of agar or gelatin are most commonly used for their cultivation.

Cultural Characters of Molds

Media adapted for mold growth are of many types, including not only the common laboratory media, particularly those useful for yeasts, but also many substances such as breads, fruits, grains, and other foods and food stuffs.

The study of a mold culture should include a determination of the *size of the colony* and of its *rapidity of growth.* The *general appearance*, whether velvety, smooth, cobwebby, cottony, etc., should be noted. An attempt should be made to see the spore-bearing organs with the unaided eye. The *color* of the mold and each of the separate parts should be recorded. Any *changes* in the *color* or *consistency* of the medium on which it is growing should be carefully noted.

CHAPTER XIV

STUDY OF PHYSIOLOGICAL CHARACTERS

All of the changes brought about by the growth of microorganisms may be termed *physiological characters*. A more extended discussion of the changes produced by bacteria, yeasts, and molds will be taken up under the heading of physiology. It is the intention in this chapter to discuss merely the methods that are used in the detection of changes.

Acid Production. — Many bacteria when grown in nutrient solutions, particularly those containing carbohydrates, produce acids and bring about thereby marked changes in *hydrogen ion concentration.*

Determination of Hydrogen Ion Concentration. — In the previous discussion of methods of adjusting the reaction of a medium it was noted that the amount of change in hydrogen ion concentration (true acidity) depends upon several factors, the most important being the following: first, the amount of acid produced; second, the kind of acid produced; third, the amount of buffer present in the nutrient medium; fourth, the amount of alkali (that is, the concentration of hydroxyl ions) produced at the same time. Alkali may be developed either by the production of ammonia or by the transformation of the salt of a strong acid to the salt of a relatively weak or little dissociated acid. For example, certain bacteria may transform sodium citrate into sodium carbonate, the latter being decidedly alkaline in reaction.

Several methods are in common use for detecting changes in hydrogen ion concentration. One of these is electrometric. This involves a rather complicated setup for a measurement of the increased electric potential of the solution due to the production of hydrogen ions. For much laboratory work simpler, though perhaps slightly less accurate, methods are to be preferred. Another is the

colorimetric method. For usual laboratory routine the colorimetric method is the simpler, and is the only one which will be discussed. In this method, it is customary to add a suitable indicator either to the solution to be tested or to a portion of this solution diluted somewhat with distilled water. The color secured is then compared with the color produced by similar addition of indicator to standard solutions whose hydrogen ion concentration is known. The indicators most used in the bacteriological laboratory for this purpose are those developed by Clark and Lubs. In the following table the name of each of these indicators is given, followed by the color in its acid range, next the color in its alkaline range, and finally the range of pH values through which it changes color.

Color Changes of Clark and Lubs Indicators

Indicators	Full Acid Color	Full Alkaline Color	Sensitive Range. The Indicator Changes from the Acid Color to the Alkaline Color between the Following pH Values
Thymol blue (Acid range)	Red	Yellow	1.2–3.8
Brom phenol blue	Yellow	Blue	3.0–4.6
Methyl red	Red	Yellow	4.4–6.0
Brom cresol purple	Yellow	Purple	5.2–6.8
Brom thymol blue	Yellow	Blue	6.0–7.6
Phenol red	Yellow	Red	6.8–8.4
Cresol red	Yellow	Red	7.2–8.8
Thymol blue (Alkaline range)	Yellow	Blue	8.0–9.6
Phenolphthalein	Colorless	Red	8.0–9.6
Cresolphthalein	Colorless	Red	8.2–9.8

It is apparent that an approximation of the hydrogen ion concentration may be secured by using different indicators and determining which gives an acid color, and which an alkaline color. For example, if it is found that phenol red gives a yellow color, the medium is acid and must be below the pH value of 6.8. If methyl red, on the other hand, gives an alkaline color, it must be of a pH value above 6. The exact pH value can be determined then by the

use of brom thymol blue, comparing the intensity of the color change from yellow to blue with standard solutions whose pH values are known.

Differences in the hydrogen ion concentration produced by different species of bacteria are frequently of use in their recognition. This is the basis of the *methyl red test* which will be discussed later as a test useful in the differentiation of certain bacteria of importance in determining the suitability of water for drinking.

For discussion of the methods of preparing standard solutions and colors for accurate determinations of hydrogen ion concentrations a suitable laboratory manual should be consulted.

Determination of Acid Production. — What has been stated above concerning determination of hydrogen ion concentration will indicate the method of determining whether or not acid has actually been produced by an organism. It should be noted, however, that the determination of hydrogen ion concentration is not a determination of the total amount of acid produced. This can be discovered only by a comparative titration. It is customary to titrate to a definite tint a sample of the sterile medium retained as a check, using phenolphthalein or some other suitable indicator. For this purpose it is usual to place 5 cc. of the sterile material mixed with 45 cc. of distilled water in a porcelain evaporating dish, add a drop or two of alcoholic solution of phenolphthalein and add twentieth normal $\left(\frac{N}{20}\right)$ alkali until a definite pink color has been established. This is kept as a standard, and the amount of alkali required noted. A similar titration is made using the medium in which the organism to be studied has been grown. This is treated in exactly the same fashion and the twentieth normal alkali added until the same tint has been secured as with the check. The differences between the amounts of alkali required for bringing to the same tint of red will vary directly with the amount of acid produced. This may be calculated in grams per liter if the kind of acid formed is known.

This method of acid determination is not absolutely accurate inasmuch as there may be basic substances developed simultaneously with the formation of acid.

In some cases it is desirable to determine the kind of acid which has been produced. Simple chemical tests for the various organic acids have not in most instances been developed. Something of their nature, however, may be determined by separating them into volatile and non-volatile. If a solution containing an acid is acidified with sulphuric acid and the materials distilled, certain acids, particularly acetic, propionic and butyric will pass over, while other acids, particularly lactic, will not. This makes it possible by titration of the distillate to determine the relative proportion of volatile and non-volatile acids.

FIG. 98. — Gas formation in dextrose agar shake culture.

Gas Production. — Gas is produced as a result of fermentation of carbohydrates by most species of yeasts, by many species of bacteria, and by a few species of molds. It is a very important means of differentiating related species of organisms. A few bacteria can also produce gas from proteins. The gases commonly produced by bacteria are carbon dioxide and hydrogen, and more rarely nitrogen or methane. The ability to produce gas may be tested by inoculating a tube of liquid agar or gelatin containing a suitable carbohydrate with the organism to be studied. If gas is evolved, the medium will be broken by numerous bubbles (Fig. 98). For a quantitative determination, it is customary to use a fermentation tube. The closed arm of this tube is entirely filled and the open arm partially filled with a liquid medium containing the carbohydrate under investigation. After sterilization, the

organism may be inoculated into the open arm, and any gas produced in the closed arm will collect in that arm. The amount of gas which forms may be determined by the use of a Frost gasometer (Fig. 99). An approximate determination of the composition of the gas produced may be made by filling the open arm with normal sodium hydroxide and covering the opening with the thumb or with a bit of rubber tissue held in place by the thumb, using care

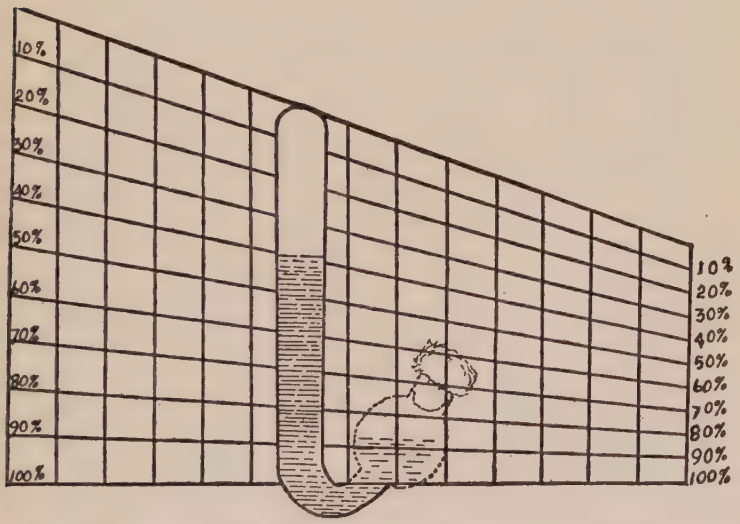


FIG. 99. — Gasometer (Frost's). The amount of gas produced in the fermentation tube is read off directly from the gasometer.

that no air bubbles are included. The gas which has collected in the closed arm may now be introduced into the open arm and shaken vigorously in contact with the alkaline solution. After the lapse of a few minutes, it may be returned to the closed arm and the thumb removed. Any *carbon dioxide* present in the gas will be absorbed by this procedure, and the liquid will rise in the closed arm to replace it. The gas remaining, if any, is usually *hydrogen*. Its presence may be demonstrated by allowing it to pass into the open arm and bringing it near a flame, when a slight explosion will occur. Rarely the gas remaining after the absorption of the carbon

dioxide may be *methane*. Some of the denitrifying bacteria produce *nitrogen* only. The *ratio* of carbon dioxide to hydrogen in the gas produced by bacteria is sometimes used as one of the differentiating characters between species. By varying the carbohydrates used in the medium, specific differences in fermentative power may often be demonstrated. The carbohydrates most commonly employed for this purpose are dextrose, lactose, and saccharose. For careful work some twelve or fifteen other carbohydrates and higher alcohols are used.

Alcohol Production. — Ethyl alcohol is produced by a few bacteria and molds, and by most yeasts. Small quantities of amyl, butyl, and propyl alcohol are also formed by a few microörganisms. Alcoholic fermentation occurs in solutions containing suitable carbohydrates, and is accompanied by the evolution of carbon dioxide. A simple qualitative test to determine the presence of alcohol in a solution is carried out by adding to a few cubic centimeters of the liquid in a test tube a small crystal of iodine and several cubic centimeters of sodium hydroxide solution. This should be heated over the flame of the bunsen burner, when, if alcohol is present, the odor of iodoform may be readily detected. Quantitative estimations are made by distillation, followed by determination of specific gravity of the distillate.

Aldehydes. — Certain microörganisms, particularly when growing in carbohydrate solutions, produce aldehyde in sufficient quantity to give a distinctive reaction. Its detection is best accomplished by the use of basic fuchsin, decolorized by the addition of sodium sulphite (or better sulphurous acid) until the color has just disappeared or until the material is of a very light pink color. When this is added to a solution in which aldehyde has developed the color of the fuchsin is restored. This is the principle made use of in the so-called Endo agar medium. Certain bacteria when growing upon this medium form red colonies because the fuchsin is freed owing to the development of aldehyde and acid. Other species of bacteria grown upon this medium do not change the color at all.

Acetyl Methyl Carbinol (Acetoin). — This compound is produced by certain bacteria growing in the presence of carbohydrates.

It is recognized by the addition to the medium of strong alkali such as sodium hydroxide or potassium hydroxide. When allowed to stand for a few hours an eosin pink or red color will develop, particularly near the surface, providing there is some peptone present. This is frequently called the *Voges-Proskauer reaction*, after the men who first noted it.

Reduction of Nitrates to Nitrites. — Certain microörganisms, particularly bacteria, when grown in a nutrient solution in the presence of nitrates, reduce the nitrates to nitrites. This may sometimes aid in the differentiation of species. The change is evidently a reduction, the organism probably making use of the oxygen obtained in this way. For determination of nitrate reduction a broth is prepared containing 0.1 per cent peptone and 0.02 per cent potassium nitrate. The inoculated tube is allowed to stand for four days, and is then tested for nitrites by means of the following solutions:

a.	5 N acetic acid	1000 cc.
	Sulphanilic acid	8 gm.
b.	5 N acetic acid	1000 cc.
	Alpha amido naphthalene	5 gm.

Two cubic centimeters of each solution are added to the tube to be tested; if nitrite is present, a red or rose color will develop. An uninoculated tube should always be tested at the same time as a check or control.

Reduction of Sulphates. — Some organisms reduce sulphates to sulphides. This transformation may be detected by the addition of an iron salt, as the chloride, to the solution, when the black iron sulphide will be precipitated, or the medium may be heated and lead acetate paper exposed to the vapor. Hydrogen sulphide will cause this to blacken. Lead acetate is also frequently added to media to detect the formation of sulphides.

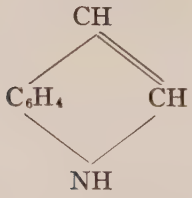
Reduction of Pigments, etc. — Under anaërobic conditions certain dyes, as methylene blue, and indicators, as litmus, may be decolorized by the growth of microörganisms. This fact is utilized in the determination of the putrescibility of sewages by adding methylene blue to a bottle filled with the sewage and corking it tightly to prevent access of oxygen. The time which elapses before

the blue color disappears is inversely proportional to the amount of organic matter which will undergo decomposition readily. The litmus added to certain media, as milk, is also frequently decolorized by bacterial growth.

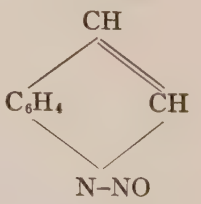
Production of Ammonia. — Many organisms, when living in a medium rich in proteins or peptones, produce ammonia. A qualitative test for ammonia may be carried out by heating the medium in which the organism has been growing and exposing a bit of filter paper dipped in Nessler's solution to the vapor. It will be turned red, brown, or black. When ammonia is produced in considerable quantities, an approximation of the amount may frequently be obtained by titration of the medium against deci-normal (tenth-normal) acid.

Indol Production. — Indol is formed by many species of bacteria when grown in a medium rich in protein or peptone and containing little or no sugar. It is customary to test for indol production by growing in peptone water or Dunham's solution. The tube should be incubated for several days before being tested. The qualitative test for indol is carried out by the addition of a cubic centimeter of 0.1 per cent solution of sodium nitrite and a few drops of sulphuric acid. The nitrite is decomposed by the action of the sulphuric acid and nitrous acid is freed. This unites with the indol to form a red compound known as nitroso-indol. Numerous other color reactions for the recognition of indol have been developed, some of them much more delicate than the foregoing. The production of indol furnishes a ready method of differentiating certain species of bacteria. The structural formulæ of indol and nitroso-indol are as follows:

Indol



Nitroso-indol



Digestion of Starch. — Many organisms, when grown in a cultural medium containing starch, form sugars or substances even

less complex. The progress of this digestion may be noted from time to time by removing small amounts of the medium and adding to these a drop of a dilute solution of iodine. The presence of starch is indicated by the blue or purple color which develops. Tests for the formation of simpler sugars may be made by the use of Fehling's solution. Many organisms which digest starch do not form any sugar during the process, or if sugar is produced at all, it is utilized and disappears almost at once.

Digestion of Gelatin. — Microörganisms that digest or liquefy gelatin bring about this change through the agency of an enzyme called *gelatinase*. The ability to liquefy gelatin is an important characteristic in the differentiation of groups of microörganisms, particularly certain of the bacteria. Recent work would indicate that many bacteria which can liquefy gelatin do not break it down into simpler compounds such as the amino acids and ammonia. In other words, some organisms apparently produce gelatinase only, others produce a trypsin-like enzyme as well.

Digestion of Blood Serum. — Some organisms are able to digest or liquefy coagulated blood serum. This change is brought about through the activity of a pepsin-like enzyme. This property is not as common as the ability to digest gelatin.

Digestion of Casein. — Many organisms when grown in milk first coagulate it, and later digest the casein. All milk cultures must be carefully examined to determine whether such digestion of casein takes place.

The digestion of casein may also be conveniently studied by means of casein agar plates. A small amount of casein solution added to agar renders the medium somewhat opaque. Colonies of organisms capable of digesting casein when grown in this medium show a clearing, the medium becoming transparent immediately about the colony.

CHAPTER XV

METHODS OF MICROSCOPIC EXAMINATION

Optics of Oil Immersion Lens. — It is necessary to use higher power objectives for the examination of bacteria and related micro-organisms than are ordinarily used for other microscopic objects. The one most commonly used for this purpose is the $\frac{1}{12}$ inch or

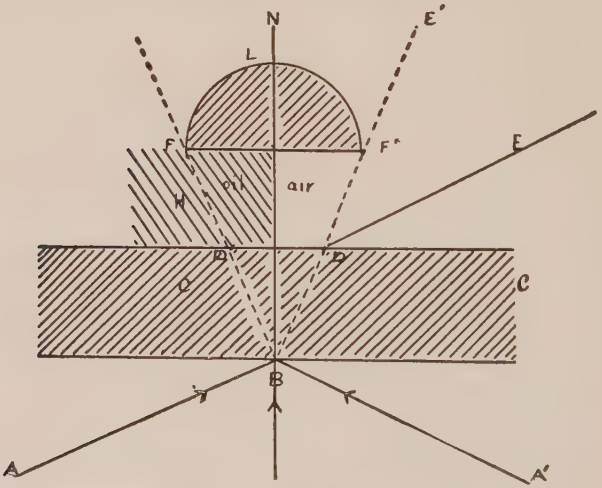


FIG. 100. — Oil immersion lens, and object glass in diagrammatic longitudinal section. See text for lettering.

1.8 to 2 mm. homogeneous oil immersion objective. A drop of oil is placed on the cover glass over the object to be examined, and the objective lowered until it is immersed in the oil. This immersion oil must always be used with a lens of this type. The following explanation of the reason for using the oil may be given. Consult the diagram in Fig. 100. Let *C* represent the glass micro-

scopic slide, L the lens at the end of the objective having an opening between F' and F, and H a drop of oil lying between the objective and slide. This oil should have the same refractive index as the glass used in the slide. The rays of light are focused by means of the mirror and Abbé condenser, being concentrated at the point B. It is evident that any ray of light, such as BN, which strikes the slide at right angles, passes straight through and enters the objective. Another ray, such as AB, striking at a considerable angle, is refracted on entering the glass in the direction BD, and on reentering the air passes in the direction DE. Such a ray does not enter the lens. Consider, on the other hand, the ray $A'B$, which is refracted as BD', and enters the oil H. It is not refracted here and passes in the direction $D'F$. This ray can enter the lens, resulting in a more brilliantly illuminated field and a clearer definition.

Preparation of Non-permanent (Unstained) Mounts

Microörganisms are frequently examined under the microscope in masses as living colonies or cultures. Mounts of a non-permanent character for more careful and critical examination may also be prepared.

Direct Microscopic Examination of Colonies and Cultures. — Cultures growing on agar or gelatin plates may be studied by inverting the plate and examining under the low power objectives. When possible, it is better to remove the cover of the petri dish and examine without inversion. The characters to be noted in bacterial colonies under these conditions have already been discussed. Molds should always be examined in this way. Frequently the arrangement of spores on conidiophores, and the branching of the latter, may be better determined by this method than by the preparation of mounts. This is particularly true in those forms in which the conidia are easily detached from their conidiophores. The branching and arrangements of the mycelium may also be observed. Higher power objectives may be used in this direct examination if a drop of alcohol or water is placed on the spot to be examined and a cover glass carefully lowered into place. Alcohol removes the air and prevents formation of bubbles, and it

is not as apt to cause spores to fall from their stalks as is water. In many cases, better views of mold-spore arrangement may be secured in this way than by any other method. Germination of mold spores may be readily studied by sprinkling the spores to be examined on the surface of agar or gelatin in a petri dish. The germinated spores may be located after the lapse of a suitable period and examined under the higher powers after covering with a glass slip.

Temporary Mounts. — Microörganisms are frequently examined by placing a small quantity of the culture in water, physiological salt solution, or broth on a microscopic slide, and adding a cover glass. If a mold is to be examined, it is often advisable to mount in alcohol first to remove air bubbles. A satisfactory method in the routine study of molds is to place a drop of alcohol on a microscopic slide and tease out the mold hyphæ added to it with two needles. A drop of dilute aqueous solution of eosin is added, the mixture allowed to stand for a few seconds, and a cover glass dropped on. The excess stain is removed by drawing it from under the cover glass by means of a bit of filter paper, water being added from a pipette on the opposite side.

All microörganisms are more or less transparent, and owe their visibility under the microscope to differences in refractivity between their cell protoplasm and water. With certain of the smaller bacteria in particular, these differences are so slight that considerable care must be used in adjusting the light by means of the iris diaphragms of the substage and Abbé condenser.

Temporary mounts of this character may be protected from too rapid drying by ringing the cover glass with vaseline.

Hanging Drops. — For the determination of motility in the bacteria, and for the continuous observance of the growth of an organism, it is customary to use the hanging drop. Motility may also be studied in a mount such as was described under the preceding heading. A hanging drop is prepared by placing a drop of the material to be examined upon the center of a cover glass. This is inverted and lowered over the cavity of a hollow ground slide and ringed with vaseline. It is usually best to focus first upon the margin of the drop, as this is more readily visible.

Preparation of Stained Mounts

Objects of Staining. — As has been noted above, it is usually difficult to see unstained bacteria. The primary object of staining is therefore to render organisms more plainly visible. It is of importance also in some cases in revealing structures such as granules within the cell or peculiar construction of the cell walls. Staining also assists in the recognition of capsules and flagella when such are present.

Stains used. — Most of the stains used for bacteriological work are aniline dyes, so-called because of their derivation from aniline ($C_6H_5NH_2$). It is customary to divide these into basic and acid stains. Bacteria are usually stained by the basic stains; certain of the acid stains are useful in studying the molds. Aniline dyes are of practically every known color. Those most commonly used are gentian violet, methylene blue, fuchsin, Bismarck brown, and eosin. The materials used for staining may be placed in two groups, the mordants and the stains proper.

Mordants. — A mordant is any material which will *fix* a stain; that is, will cause an organism to stain more deeply or retain the stain more firmly than if it were not used. Phenol or aniline when added to certain staining solutions render them more intensive in their action. Mixtures of potassium iodide and iodine, of tannic acid and iron sulphate, and other solutions are used as mordants for particular purposes.

Formulæ of Stains most commonly used. — The following stains are in common use in the laboratory. For special purposes a great number of others have been described.

Loeffler's Methylene Blue

Saturated alcoholic solution of methylene blue	15 cc.
Solution of potassium hydroxide $\frac{1}{1000}$	45 cc.

Aqueous Solution of Gentian Violet

Saturated alcoholic solution of gentian violet	2.5 cc.
Distilled water	47.5 cc.

Aniline Gentian Violet (Ehrlich's)

Saturated alcoholic solution of gentian violet	6 cc.
Absolute alcohol	5 cc.
Aniline water	50 cc.

Aniline water may be prepared by shaking 2 cc. of aniline with 98 cc. of water for several minutes. It should then be filtered through paper until clear. This is practically a saturated solution in water.

Carbol Fuchsin

Saturated alcoholic solution of basic fuchsin	5 cc.
Solution of phenol 0.5 per cent	45 cc.

Bismarck Brown

Saturated aqueous solution

Eosin

Water-soluble eosin	0.5 gm.
Distilled water	100 cc.

Preparation of Stained Mounts. — Stained mounts of bacteria and yeasts are usually prepared by the following method. A small drop of water is placed upon a thoroughly clean cover slip. By means of a sterile platinum wire or loop, a small portion of the culture to be examined is mixed in the drop of water and spread over the surface of the glass in a uniform layer. If the glass is perfectly clean, there will be no tendency for the water to round up in a drop, but it will adhere to the glass and spread uniformly. When the organism is growing in a liquid medium, the water may ordinarily be dispensed with and the cover-glass preparation made directly with a drop of the nutrient solution. This material is then allowed to dry in the air, or it may be held at a distance above the bunsen burner between the fingers in such a way that it will not be overheated. As soon as perfectly dry, it is *fixed* by passing film side up two or three times through the flame of the bunsen. This causes the organisms to adhere firmly to the glass, so that they are not easily displaced by later treatment with stains and other reagents. After fixing, the preparation is ready for staining. A drop of the stain to be used is placed upon the surface and allowed to act for a period from a few seconds to as many minutes or even longer, depending upon the organism to be examined and the stain used. In a few cases, it is necessary to keep the stain warm by holding above the bunsen flame until it steams and by replacing the stain as rapidly as it evaporates. The

cover slip is then washed in running water until no more stain comes off. It may then be dried by blotting between filter paper. In examination, it is customary to place a drop of water on the glass side, and lay the cover slip, film down, upon this. If microscopical examination shows the mount to be satisfactory, it may be made permanent by floating off the cover glass by means of a small drop of water, drying both slide and cover glass carefully, placing a drop of Canada balsam on the slide, and pressing down the cover glass, film side downward, upon it.

In routine work in the laboratory, the cover slip is frequently dispensed with, the smear being made directly upon a glass slide. The process of fixing and staining is carried out in the same manner as noted above. After drying the immersion oil is placed directly upon the film and examination is made without the use of the cover glass.

It is sometimes necessary to use other methods of fixing than heat. For some purposes it is customary to immerse the film in absolute alcohol or expose it to the vapor of osmic acid.

Bacterial Spore Stain. — The spores of bacteria do not ordinarily stain as readily as do the vegetative rods, but when once stained, are not as readily decolorized. Hansen's method is the simplest of the many proposed, and will yield most satisfactory results.

1. Prepare a film of the spore-producing organism. Dry, fix with heat, and stain with steaming hot carbol fuchsin for five minutes. The stain is heated directly upon the cover glass or slide by holding it above the bunsen flame until vapor is seen to arise. It is then moved to one side and the process repeated as soon as vapor ceases to be given off.
2. Decolorize with 5 per cent acetic acid until the film is light pink. The time necessary for this will depend upon the preceding treatment and upon the density of the film; a few seconds is usually sufficient. Then wash in water.
3. Stain for three minutes with Loeffler's methylene blue.
4. Wash in water, dry, and examine.

The spores should be stained red and the bodies of the vegetative rods should be blue.

Flagella Stain. — The flagella of bacteria are not visible in stained mounts as usually prepared. Special methods are necessary for their demonstration. Young, actively motile cultures, preferably those not more than twelve to eighteen hours old, should

be used. Agar slants are perhaps most satisfactory. A tube containing 5 cc. of sterile water or physiological salt solution is inoculated with a sufficient quantity of growth from the agar slant to produce slight turbidity. This is then kept for an hour in the thermostat at 37.5° C. Two or three drops are placed on a clean cover glass and allowed to dry without mixing or spreading. This is fixed in the flame. Loeffler's method of staining will give very satisfactory results when carefully carried out. Many other methods have been devised.

1. A water bath containing boiling water should be prepared.
2. Prepare the film and fix as already indicated.
3. Apply the following mordant; heat for five minutes over the steaming water bath:

Tannic acid (25 per cent aqueous solution)	10 parts
Saturated solution of ferrous sulphate	5 parts
Fuchsin, saturated alcoholic solution	1 part

4. Wash in water and blot with filter paper.
5. Stain with aniline gentian violet or carbol fuchsin over the water bath for five minutes.
6. Wash and examine.

Much care must be used in all details of the process, and repeated trials are often necessary before a satisfactory mount is obtained.

Gram's Staining Method. — Gram's staining method was first used in the demonstration of bacteria in diseased tissues. By this method certain bacteria were found to retain the stain, while the tissues in which they were imbedded lost the stain. It was afterwards discovered that bacteria could be divided into two groups, those which would retain the stain when treated by this method, the so-called gram-positive forms, and those which would lose the stain, the gram-negative forms. A statement as to the gram staining characters of an organism is practically always included as a part of its description.

1. Prepare a film; dry and fix.
2. Stain one and one half minutes in aniline gentian violet.
3. Treat with iodine solution one and one half minutes. This has the following composition:

Iodine	1 gm.
Potassium iodide	2 gm.
Distilled water	300 cc.

4. Decolorize with 95 per cent alcohol until most of the stain seems to be washed out, or immerse in the alcohol for five minutes. Wash in water, dry, and examine.

Stain for the Yeast Nucleus. — It is only within recent years that satisfactory methods of demonstrating the nucleus of the yeast have been developed. Many are now known, but perhaps the method of Wager (somewhat modified) is the simplest and as successful as any. Young actively growing cultures of the yeast on wort agar slants or from the scum of an actively fermenting sugar solution should be used. The material taken from these surfaces should be dropped into a test tube containing saturated solution of mercuric chloride in water and allowed to remain about twelve hours (or over night). The cells will then have settled to the bottom, and the liquid may be removed by means of a pipette. Water is then added, the tube shaken, the cells allowed to settle out, and the water removed. This process may be greatly hastened by using a centrifuge tube and centrifugalizing for a minute. Next the cells are washed successively (as just described) with 30 per cent alcohol, 70 per cent alcohol, and absolute methyl alcohol. This preliminary procedure is to *fix* and *harden* the protoplasm of the yeast cell and to prevent shrinking and plasmolysis. A drop of the methyl alcohol containing the yeast cells is placed upon a microscopic slide, and allowed to evaporate nearly to dryness; a drop of water is then added and spread. This is allowed to dry completely in the air. The yeast cells will now adhere to the slide sufficiently so that they may be stained with a dilute mixture of fuchsin and methylene blue, washed carefully in water, dried, and examined.

Permanent Mounts of Molds. — The simple methods that have been described for preparation of permanent mounts of bacteria and yeasts will not suffice for the molds in most instances. Molds in many cases do not require any staining. This is particularly true of those forms that have brown or smoky cell walls. These are usually mounted by placing them in a small drop of 50 per cent glycerol on a glass slide, adding a cover glass, removing carefully any excess glycerol, and ringing the cover with asphaltum or microscopic cement both to bind the cover firmly in place and to

prevent loss of glycerol. When this treatment causes a collapse of the cell, a 0.5 per cent solution of formalin in water may be substituted. In this case it is absolutely necessary to seal the mount completely or the water will quickly evaporate.

Forms that are transparent require staining to bring out details of structure. The following method may be applied successfully to molds growing on solid media, particularly on gelatin or agar in a petri dish.

Fill the dish with absolute alcohol, cover, and allow it to stand for five or ten minutes. This serves two purposes: it fixes or hardens the protoplasm of the cells and removes any air, eliminating air bubbles. A portion of the mold to be examined is removed to a small dish (as a watch glass) containing one per cent aqueous solution of eosin. The stain should be allowed to act for five minutes. Transfer then to water and wash until the color ceases to come out. A little of the material may be examined under the microscope, and if stained too heavily, it should be transferred for a few seconds to 5 per cent acetic acid, then back at once to distilled water. The permanent mount should be made in 50 per cent glycerol, as described above. Some mold hyphæ and sporangia, as those of Rhizopus and Mucor, may collapse or plasmolyze if put at once into 50 per cent glycerol. This may be obviated by placing in 10 per cent glycerol and exposing to the air to concentrate slowly by evaporation. The mold may then be mounted under a cover glass and ringed.

For methods of demonstrating nuclei and details of cell structure one should consult a text on plant histology.

SECTION III

PHYSIOLOGY OF MICROÖRGANISMS

CHAPTER XVI

EFFECTS OF ENVIRONMENT UPON MICROÖRGANISMS

GENERAL DISCUSSION

THE *physiology* of an organism may be defined as including all the inter-relationships between the organism and its environment. This would comprise all of the effects of environment upon the organisms, and all of the effects of the organism upon its environment.

The environmental factors which chiefly concern us are either physical or chemical in nature. Their effects upon the organism will be discussed in this section, the effects of organism upon environment will be treated in Section IV.

The effects which may be produced upon microörganisms by their physical and chemical environments are of many types. Physical or chemical influences may increase or decrease the rates of growth or development, they may increase or decrease the rates of death, they may bring about changes in the morphology of the organisms, they may modify the changes which the organism may produce, they may modify the rate at which an organism moves. These are a few only of the effects which may be produced. They will be discussed in the succeeding chapters.

In many cases we are interested from a practical point of view in the effects of the environment upon rates of growth and death. If under one set of conditions an organism grows slowly, and under another set it grows rapidly, we may determine the effect of environment by comparing the rates of growth or the rates of increase. Correspondingly we may compare the effects of unfavorable conditions by noting the comparative rates of death which they produce. It is apparent, therefore, that we should have methods of measuring or determining the *numbers* of organisms or the *amount* of growth.

We must also understand what is involved in the term *rate* as applied to increase or to death, and how these rates may be determined.

Measurement of Numbers of Organisms or of Amounts of Growth

There have been many methods suggested and used for determining at intervals the number of living cells present in a culture medium or of determining the total amount of growth which has occurred. Several of the methods more commonly employed will be discussed.

Determination of Numbers by Direct Microscopic Count. — Three principal methods of determining numbers of microörganisms by direct microscopic observation have been employed. In some cases (particularly with yeasts) it has been found practicable to use a modification of the counting chamber or hemocytometer employed by physicians in determining the number of red blood-corpuscles per cubic millimeter of blood. This method consists essentially in observing directly and counting the number of cells present in a given volume of the liquid.

Another method is to spread a definite small volume of the liquid containing the bacteria over a definite area of glass slide, this to be followed by drying, fixing, and staining. It is necessary to know the area of the field of the microscope used for counting. One may then count the number of bacteria or other cells present in a series of microscopic fields. It is then easy to calculate the number of cells present in the volume of liquid originally spread upon the slide. It is also possible to determine numbers by a method termed that of proportional counts. It is evident that these methods of counting bacteria or yeasts would not enable one to differentiate between living and dead cells. Such methods require modification, therefore, if they are to be adapted to the determination of the total number of living cells which are present.

Cultural Methods for Counting Living Cells. — The most common method of determining the total number of bacterial or yeast cells which may be present is by mixing a definite amount or dilution of the liquid with melted agar or gelatin, then pouring into a sterile

petri dish. When cooled the medium will solidify. The individual cells are thus fixed in place, and under favorable environmental conditions will grow into colonies which may be counted; knowing the dilutions employed in preparation of the plate, one may estimate the number of living cells in a given volume of the material. It is important that suitable dilutions be employed, otherwise the colonies may be so crowded together that they are difficult to count, indeed the growth or development of some may be altogether inhibited. This method is the one which is usually termed *plating out.*

Methods of estimating numbers by determination of the highest dilution of the liquid which may be inoculated into a suitable culture medium and produce growth have sometimes been employed.

Measurement of Increase by Other Methods. — It is sometimes practicable to determine the total amount of increase by measurement of the total volume of organisms produced, by weighing, by measuring the size of colonies, by determination of the turbidity which is produced when the organisms are grown in a liquid medium, or by measuring the amount of some product of growth. These are somewhat less frequently employed than those mentioned above. Details as to methods of utilizing them may be found in standard laboratory manuals.

Rates. — A rate is usually designated as the amount of change in a given length of time. The rate of increase in the bacterial culture, for example, for a given period of time would be the increase in the number of bacteria during that interval. A bacterial death-rate correspondingly would be the number of bacteria which die in a given length of time. It is quite evident that when bacteria are multiplying, the number of new cells produced in a given length of time will depend upon the number which are present at the beginning of that time. It is frequently advisable therefore to employ the designation rate of increase or rate of death per cell. This latter rate is frequently called the *velocity coefficient* of the rate of growth or of death. Comparisons of effects of environment are frequently made by noting the velocity coefficients of the rates of change under the respective conditions.

Growth. — It has already been noted that bacteria multiply by fission, that is, each mother cell divides into two daughter cells.

The length of time which elapses between consecutive cell divisions, that is the length of time that is required for a single cell to grow to its full size and divide to form two individuals, may be termed the *generation time*. It is apparent that the shorter the generation time the more rapidly are the bacteria multiplying. We can therefore judge of the effect of various physical or chemical influences upon the growth of an organism by noting any variations in the length of the generation time which are produced. It is usually not convenient actually to watch the microörganisms under the microscope and to determine by means of a stop watch the length of time required for cell division or the length of the generation time. Methods of counting, such as those enumerated above, have been devised so that we may know the number of living bacteria present in a cubic centimeter of liquid at the beginning of any definite period of time and the number at the end of that period. From this it is possible to calculate the length of the generation time as noted below. It must be emphasized that this must be done on the assumption that the conditions do not change materially during the period of observation, in other words, the cells should continue throughout the period to multiply regularly.

Let **n** be the number of cell divisions, that is the number of generations which develop during a given time, which we may represent by **t**. If we start with one organism we shall have at the end of one generation period two organisms, and at the end of the second generation period four organisms and at the end of the third period eight organisms. It is apparent, therefore, that the number of organisms originating from a single cell will be at the end of the first period 2^1, at the end of the second period 2^2, at the end of the third period 2^3, and at the end of the nth period 2^n. If instead of beginning with a single organism we start with any number, which we may represent by **B**, the number at the end of the nth generation period will be

$$B\ 2^n.$$

If we let the number of bacteria after time **t** be represented by **b**, we have the equation

$$b = B\ 2^n.$$

If this equation be solved for **n**

$$n = \frac{\log b - \log B}{\log 2}.$$

Since log 2 is 0.30103, this relationship may be written

$$n = 3.3 \log \frac{b}{B}.$$

that is, if one divides the number of bacteria at the end by the number at the beginning, then multiplies the logarithm of this quotient by 3.3, the result is the number of generations. It is evident that the number of generations which will develop in time **t** will be equal to the total time divided by the generation time, that is

$$n = \frac{t}{g},$$

or conversely

$$g = \frac{t}{n},$$

and

$$g = \frac{t}{3.3 \log \frac{b}{B}}.$$

It is evident that the more rapid the growth rates of a cell the smaller will be the value of **g**. One may compare the effects of environment therefore by comparing the values of **g** secured under the given conditions. The rate of increase is inversely proportional to the length of the generation period.

Rates of Death. — When organisms are placed under sufficiently unfavorable conditions they cease to multiply and begin to die. In many of the cases which have been carefully studied the bacteria die off in accordance with a definite law, which may be stated as follows: With a given kind of organism under uniform conditions, the number of bacteria present in a culture will always be reduced by one-half in equal periods of time, that is, no matter how many bacteria there are at the beginning of a definite period of time, one-half that number will always be alive at the end of the proper interval. For example, suppose that two cultures, one containing

a million bacteria and the other a thousand bacteria are subjected to the same unfavorable conditions. It is found that at the end of a definite period of time, say ten minutes, the bacteria in the less concentrated suspension have been reduced to 500. It will be found that in the same period of time the other culture has also been halved, that is, there are 500,000 bacteria left. Another way of stating is this, — during each equal interval of time a definite percentage of those bacteria living at the beginning of the period will be killed. If we wish to compare unfavorable conditions in their effect upon the death of microörganisms we may compare the length of time required to reduce the numbers of bacteria by a definite percentage, say one-half. If at one temperature, for example, half of the bacteria are killed in ten minutes, and at another temperature one-half the bacteria are killed in five minutes, it is evident that the second temperature is far more destructive than the first. It will be noted that the time required to kill half the bacteria is mathematically the converse of the generation time.

The statement above outlined does not hold good for all bacteria under all conditions. When it does, however, it is possible to compare environmental effects on death rates.

CHAPTER XVII

EFFECTS OF PHYSICAL AGENCIES ON MICROÖRGANISMS

The principal physical factors influencing growth and development of microörganisms, that is, their growth rates and death rates, are moisture, osmotic pressure, light, temperature, electricity, and mechanical pressure. However, it is not always easy to differentiate between physical and chemical environmental factors as to their effects. These physical factors will be considered in order.

Moisture. — The optimum moisture condition for most yeasts and bacteria is saturation. Molds frequently develop best with less moisture. Complete *desiccation* (drying) will kill many organisms, particularly certain of the disease-producing forms such as the typhoid bacillus. On the other hand, the spores of bacteria, yeasts, and molds may withstand drying for years. Some bacteria and yeasts that produce no spores are also very resistant to desiccation. It is difficult to determine in all cases just why drying should destroy the cell. Death in some cases is doubtless due to too great concentration of the solutes about an organism when drying and to the consequent increase in osmotic pressure. It has been found, for example, that some organisms when frozen and dried quickly in a vacuum retain their vitality for a long time, while if dried when not frozen, the cells are quite certainly killed. The freezing prevents the concentration of solutes and any increase in osmotic pressure. In other cases probably, the dyring brings about some irreversible change in the protoplasm that is not compatible with its proper functioning.

Drying stops all growth and activity of the microörganisms that may be present in any material. It is therefore of importance as a means for preservation of foods and in the prevention of decay in general.

Osmotic Pressure. — In the discussion of morphology of microorganisms, the ectoplast or outer differentiated layer of the cell protoplasm lying just within the cell wall was described as a semipermeable membrane. The normal cell generally has a higher content of solutes within its walls than is to be found in the solution in which the cell is growing. This insures that there is a greater

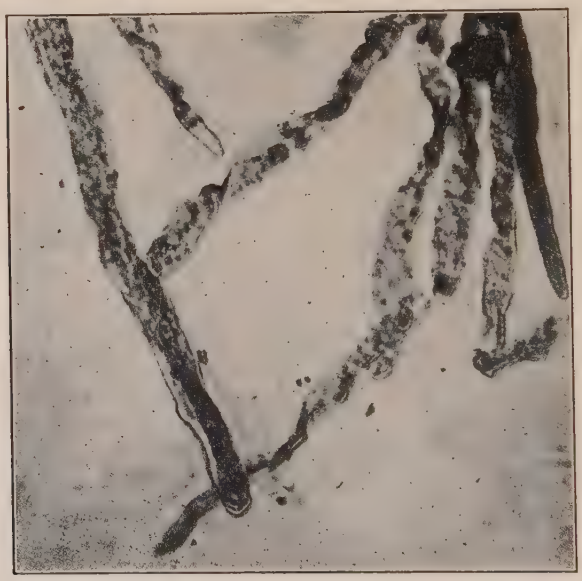


FIG. 101. — Plasmolysis of mold hyphæ. Hyphæ of Mucor which have been placed in a glycerol solution. Note that the protoplasm is withdrawn from the hyphal walls in many places and that the latter are much wrinkled and shrunken. (× 500.)

pressure inside than outside. The ectoplast is firmly appressed to the cell wall and the cell is said to be *turgid*. When a cell is placed in a solution having a higher content of solutes than has the protoplasm, and the solutes do not readily pass into the cell, conditions are reversed; water leaves the cell and it shrinks in size. If the difference is great enough, this shrinking continues until the ectoplast separates from the cell wall and the protoplasm contracts. This process is called *plasmolysis* (Fig. 101). If the difference is not too great, the cell gradually adjusts itself to the new conditions

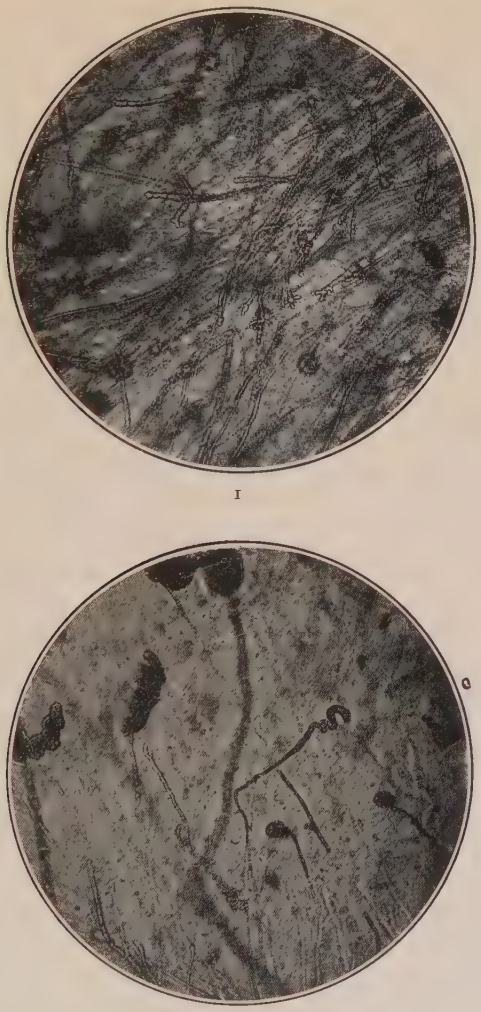


Fig. 102. — Plasmoptysis of mold hyphæ (Aspergillus). A drop of distilled water was placed at the margin of an old colony on beerwort agar whose sugar content had been greatly increased by evaporation of the water. 1, Filaments showing decided swelling of the tips due to the lowered osmotic pressure on the exterior. 2, Filaments have absorbed water until they have burst and the protoplasm has exuded. (× 300.)

regains its turgor, and continues growth. If the difference is greater, the cell remains permanently plasmolyzed and is killed. Placing a cell grown in a solution containing a considerable quantity of solutes in distilled water may bring about the reverse of plasmolysis, a swelling of the cell, sometimes followed by a bursting of the cell wall and escape of the contents. This is called *plasmoptysis* (Fig. 102).

Bacterial cells are relatively refractory to plasmolysis; it is most easily demonstrated in members of the genus Vibrio. The phenomenon is readily observed, however, in molds. The latter also show plasmoptysis clearly.

It is evident that for every solute there exists a concentration too great to permit the growth of organisms. Advantage is taken of this in the preservation of foods (as sirups, jellies, etc.). Some organisms can adjust themselves to a very high concentration of sugar, as evidenced by the development of molds on jellies and preserves. Some yeasts are known which grow by preference or only in high concentrations of sugar. They are sometimes termed *osmophilous*.

Light. — Light rays act either by inhibiting or by stimulating growth; the first of these two influences is by far the more important. It seems to be a property common to all protoplasm that injury follows exposure to intense light such as the direct rays of the sun. Sunburn of the skin in man, for example, is a direct result of such irritation. Cells are sometimes protected by membranes or pigments from being influenced thus. This is not usual among bacteria and yeasts, but is perhaps more common among the molds, particularly in the spores. Direct sunlight is therefore an efficient germ destroyer, and to its activity we probably owe much of our freedom from disease.

It has been found that not all rays of light are equally injurious to cells. The red and yellow rays are relatively inert, while the blue and violet rays are much more powerful. The spectrum extends beyond the violet and indigo into the ultra-violet, rays not recognized by the human eye. These are by far the most destructive. This germicidal action of violet and ultra-violet rays has been utilized practically in certain devices for the purification of

water. The Cooper-Hewitt mercury vapor arc light (Fig. 103) has become of considerable importance for this purpose. This lamp consists of a cylinder of glass or quartz exhausted of air, containing some mercury, and with electric terminals at the ends. The light issues from the mercury vapor arc between these two poles. Glass does not permit the ready passage of ultra-violet rays; hence it is replaced by quartz in lamps designed for sterilizing purposes. The water to be sterilized is allowed to flow past the lamp and is exposed to the rays at short range for a few seconds. The organisms present are destroyed by this exposure.

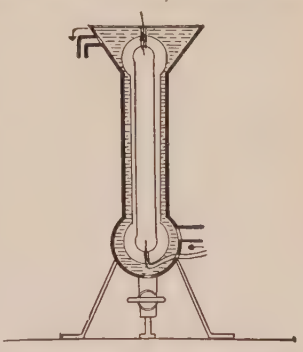


FIG. 103. — Cooper-Hewitt mercury vapor lamp adapted for sterilization of water. The water enters at the bottom and leaves at the top, passing along the side of the lamp, and is thus exposed to the rays effectively. (Adapted from Bertarelli.)

Only light which is *absorbed* by the protoplasm of the cell can injure it. It has been found that protoplasm is relatively opaque to ultra-violet light. This is one factor in explaining its destructive effect upon the cell. The mere fact of absorption, however, does not alone explain the injury. There must be a chemical effect produced. Light, particularly diffuse light, sometimes stimulates the growth of a few microöganisms, usually molds. These may grow toward light in much the same manner as does a green plant in a window. This phenomenon is called *phototropism*. Alternate exposure to light and darkness will sometimes stimulate growth and spore production in the molds in concentric bands. One group of bacteria (certain sulphur bacteria) grow regularly in the light. This is not true of most kinds of bacteria. They grow best or only in the absence of light.

Temperature. — It is a very general rule in chemistry that an increase in temperature increases the rate at which a chemical reaction takes place. Very commonly (though by no means universally) the rate doubles or trebles for each ten degree increase in temperature. Inasmuch as the life processes of a cell are largely

chemical or based upon chemical changes, it might be inferred that similarly increases in temperature would increase biological rates. In fact, within certain limits fixed for each kind of organism and set of conditions, an increase in temperature does increase the rate of growth. For every cell, however, there is apparently a temperature which yields the most rapid growth; at higher temperatures the rate of growth decreases, and at sufficiently high temperatures ceases altogether. In other words, for every living cell there is an *optimum* and a *maximum* growth temperature. Cells usually also exhibit a definite *minimum* growth temperature. The distance from the minimum growth temperature to the maximum may be termed the *growth temperature range.* These special temperatures are sometimes termed the *cardinal* points. At temperatures higher than the maximum the microörganisms are destroyed, the more rapidly the higher the temperature.

Optimum Growth Temperature. — The optimum temperature of an organism is that temperature at which it grows most rapidly, that is, that temperature at which the generation time is least. The optimum is not a fixed temperature for a particular species; it varies with changes in the environment. It may be changed, for example, by an alteration in the composition of the nutrient medium in which it is grown. Usually, however, the change in optimum temperature with variation of the other environmental conditions is not great enough to interfere with the classification of microörganisms into the three groups noted below.

Thermophilic bacteria are those that develop best at relatively high temperatures, usually above 45° to 50° C. These organisms have been repeatedly isolated from the waters of hot springs, from the interior of decaying heaps of compost or manure, from fermenting ensilage, from soil, and from the intestinal contents of man and animals. Many of them are spore-bearing bacilli. Few of them are of much practical importance.

Psychrophilic organisms are those that grow best at relatively low temperatures, usually below 10° C. They are commonest in cold waters such as those of springs, wells, and the depths of lakes or the ocean. Some of these may be of importance in the decay of foods in cold storage.

Mesophilic organisms are those whose optimum temperatures are between these two extremes. They may be divided into two groups, those found in the bodies of man and animals in health or disease and having an optimum temperature of about blood heat (37.5° C.) and those having optima somewhat lower. The decay-producing and putrefactive bacteria frequently have optima between 20° and 35° C. Yeasts, in general, grow best between 20° and 30° C., and molds have similar or even lower optima.

It should be noted that the optimum temperature is not necessarily the temperature at which the organism will bring about a maximum amount of change. Less acid, for example, may be produced at the end of a certain period by an organism kept at its optimum than when at a lower temperature. It will later be noted that the efficiency of antiseptics depends in part upon temperature; a small amount of acid at a high temperature may have a more deterrent effect upon growth than a larger amount at a low temperature.

Minimum Growth Temperature. — The *minimum* growth temperature of an organism is the lowest temperature at which growth will occur. The minimum for most true thermophiles is above 40° C. The minimum for some pathogenic bacteria is but two or three degrees below the optimum. In most cases, however, it lies much lower, usually at 8° to 10° C. Some can develop at still lower temperatures. Freezing at once stops multiplication in all cases, probably because ice is dry, and the effect is much as in desiccation. When solutes are present in sufficient quantity, water may be lowered to a temperature considerably below 0° C. without freezing. Under these conditions some organisms continue to multiply slowly. It is evident, then, that some foodstuffs cannot be indefinitely preserved at a temperature above the point of freezing of the liquids contained.

The *maximum* growth temperature is the highest temperature at which growth and multiplication can take place. In most cases, but by no means in all, the maximum temperature is but a few degrees higher than the optimum. The maximum temperature for some of the thermophilic bacteria is nearly 80° C. This temperature is so high that we must think of their protoplasm as

differing from that of other cells, for most native proteins coagulate on exposure to this temperature. The maximum for most pathogenic bacteria lies between 40° and 50° C. Growth at high temperatures sometimes causes a decrease in virulence or disease-producing power. The maximum for many yeasts is between 30° and 40° C. Very few molds develop well at body heat.

The *growth temperature* range of an organism is the number of degrees difference between the minimum and the maximum. This is very small with some bacteria, particularly some of the pathogenic forms. Some will not develop unless kept within two or three degrees of body temperature; in most cases, however, the range is much broader.

Freezing does not commonly destroy microörganisms at once. When frozen, they gradually decrease in numbers. Temperatures lower than freezing do not seem to have any additional effect. Milk containing lactic acid bacteria may be exposed to the temperature of liquid air, and when thawed and kept for a time at room temperature, will sour normally. Sterilization of food products by freezing is therefore not practicable. The gradual decrease in numbers of living organisms in frozen materials is of considerable importance in that it is undoubtedly one of the safeguards in the use of ice. Bacteria, particularly the disease-producing forms, do not persist indefinitely. This decrease is even more striking with frozen milk products such as ice creams.

Thermal Death Point. — A thermal death point is sometimes defined as that temperature which in a given length of time will kill a particular organism. This definition, however, is hardly adequate. We have seen that all the organisms in a culture do not die instantaneously upon being subjected to unfavorable conditions, but that under a definite set of conditions, there will be a definite rate of death. Theoretically it would be better to designate the *rate of death* under certain standard conditions at a definite temperature, rather than to use the term "thermal death point." It has also been suggested that the term thermal death time be used to designate the length of time required to kill all the cells at a given temperature. The rate at which bacteria die off at a given temperature is influenced by several factors.

First, the *time of exposure* must always be noted. A lower temperature for a longer time may be as efficient as a higher temperature for a shorter time. This matter is of importance in the sterilization and pasteurization of foods where it is necessary to kill all bacteria or all bacteria of certain types, but where heating to too high a temperature will injure the flavor. The time commonly used in thermal death point determinations is ten minutes, and where time is not specified, this is usually understood. It is evident from preceding discussions that the time of exposure necessary to sterilize is also dependent upon the number of bacteria present in the beginning. It will take a longer time to kill all of a large number of bacteria than all of a smaller number.

Second, the thermal death point, or rate of death, will depend upon the amount of moisture present. Moist heat is much more efficient than dry heat. Probably the explanation for this is to be sought in the difference in coagulation temperatures of moist and dry proteins. The albuminous protoplasm of the cell is not readily coagulated when dry. This fact is well illustrated by the following table from Frost and McCampbell's *General Bacteriology*.

Egg albumen + 50 per cent water coagulates at 56° C.
Egg albumen + 25 per cent water coagulates at 74–80° C.
Egg albumen + 18 per cent water coagulates at 80–90° C.
Egg albumen + 6 per cent water coagulates at 145° C.
Egg albumen + 0 per cent water coagulates at 160–170° C.

It is evident therefore that in the sterilization of dry objects, as laboratory glassware, it is necessary to use a relatively high temperature as 140°–150° C. for an hour, while moist heat, as in the autoclave, is even more efficient at 120° C. for ten minutes. Boiling in water will kill everything but the most resistant of spores.

Third, the *reaction and composition* of the medium in which the organism is heated has a marked influence on the thermal death point. It is much easier to sterilize an acid fruit, such as the strawberry or tomato, than the more nearly neutral vegetables, such as peas or corn. Acidity in particular renders heat much more effective. It is necessary in comparative work, therefore, to use media having uniform reaction and composition. The term acidity as here used, means, of course, the actual acidity, or

better the concentration of hydrogen ions. Other substances may also be important. For example, the thermal death rate of bacteria is lower in cream than in whole milk, and lower in the latter than in skim milk.

Fourth, the *presence of spores* indicates that the organism has in fact two thermal death points: one for the spores and the other and lower one for the vegetative cells. Boiling for an hour will not certainly destroy all spores. Use of the autoclave and temperatures of 110°–120° C. are necessary if they are to be killed in a short period of time. The spores of certain of the thermophilic bacteria are particularly difficult to destroy. They are of considerable importance, as in the commercial canning of corn.

Fifth, the *specific character of the organism* must be taken into consideration. There are evidently intrinsic differences in the protoplasm in different species. Many organisms are destroyed rapidly at temperatures of 55°–60° C. Others require much higher temperatures. The careful determination of the thermal death point for all pathogenic bacteria is evidently desirable, for upon this determination must rest the efficiency of pasteurization of milk in preventing the spread of disease.

It should be again emphasized that microörganisms held at temperatures above the maximum growth temperature are dying and that the rate of death increases rapidly with the increase in temperature. It has been found with spores that the death rate is increased five to eight times by each increase of ten degrees in temperature; for example, if a temperature of 100° C. for 30 minutes is required to kill a certain number of spores, a temperature of 110° C. for less than 6 minutes would probably prove equally effective.

It may be noted that sometimes a practical sterilization by heat may be effected without actually destroying all the bacteria present, providing the nature of the medium in which the organisms are present or the conditions under which it is kept are such as to prevent growth.

Mechanical Pressure. — Several attempts have been made to destroy bacteria by means of mechanical pressure. For example, the attempt has been made to kill the bacteria by filling tin cans

with food to be preserved, then immersing the sealed cans in water and subjecting to hydraulic pressure. It was not found practicable to apply pressures sufficiently high to kill all the microörganisms present, particularly the spores of bacteria. It has been found possible by use of pressure of many thousands of pounds to the square inch to kill non-sporulating organisms. It is possible that this method may find application in the preparation of vaccines.

Electricity. — Electricity has apparently little direct effect upon bacteria; the passage of an electric current of low voltage through a suspension of bacteria is not directly injurious to the cells. Under certain conditions, however, the electric current may bring about the formation of compounds that act as disinfectants. Advantage is taken of this fact in certain cases in the sterilization of sewage effluents or other solutions containing the chlorides of sodium, calcium, or magnesium. When a current of sufficient tension (at least 2.5 volts) is passed through such a solution, chlorine gas appears at one electrode, alkalies at the other. This process of electrolytic disinfection has proved to be a relatively efficient method of freeing water from microörganisms.

CHAPTER XVIII

RELATIONSHIP OF MICROÖRGANISMS TO CHEMICAL ENVIRONMENT

The chemical constitution of the medium in which an organism is growing affects it in many ways. It may influence the rate of growth by supplying essential nutrients, it may stimulate or retard growth, it may determine the total amount of growth, it may affect the direction and rate of movement of motile cells, it may alter the ability to produce disease or modify the substances produced by growth. Certain of these effects are of considerable practical significance, and may be discussed here.

Relationship of Free Oxygen to Growth of Microörganisms. — Some microörganisms will not grow in the absence of free oxygen, others will not develop in its presence, while still others can adapt themselves to either condition. An organism which requires free oxygen is termed *aërobic*, one whose growth is inhibited by oxygen is *anaërobic*, while one that grows under aërobic or anaërobic conditions is called *facultative*. The lines separating these groups are often not well marked. Some organisms will neither grow in contact with oxygen at a pressure identical with that of the air, nor in a medium totally devoid of oxygen. Such are sometimes termed *microaërophiles*. It is important to determine the concentration of oxygen necessary in such cases. Sometimes (particularly by those working with protozoa) the terms *oxybiontic* and *anoxybiontic* are used respectively for organisms which require and do not require free oxygen.

All organisms must secure energy for growth and movement through transformations of chemical compounds. This securing of energy from food is termed *respiration*. In many cases this consists of an actual oxidation of the food substances by means of

atmospheric oxygen; in others it results from rearrangement of atoms within a molecule, or a breaking up of the molecule. The latter may be termed an intramolecular oxidation. These methods of securing growth energy may be termed *aërobic* and *anaërobic* respiration respectively. They may be illustrated by two common changes brought about by microörganisms: the aërobic by the oxidation of dextrose to carbon dioxide and water, and the anaërobic by the transformation of dextrose into lactic acid.

$$\underset{\text{Dextrose}}{C_6H_{12}O_6} + 6\ O_2 = 6\ CO_2 + 6\ H_2O$$

$$\underset{\text{Dextrose}}{C_6H_{12}O_6} = 2\ \underset{\text{Lactic acid}}{C_3H_6O_3} \text{ or } 2\ CH_3.CH(OH).COOH$$

It is evident that more energy is secured by the first process than by the second, for in the first there is complete oxidation and in the second oxidation is accomplished by a rearrangement of the elements. In the production of lactic acid from dextrose there is reduction of some carbon and oxidation of other. The ratio of energy or heat production in the two cases is about 674:15. In other words, the transformation of dextrose into CO_2 and H_2O yields forty-five times as much energy as the change into lactic acid, and an organism securing its energy by the second method requires forty-five times as much food as one using the first. This indicates the reason why organisms living under anaërobic conditions require so much greater quantities of food for a given amount of growth than do aërobic forms.

Some bacteria can grow under anaërobic conditions, provided certain compounds are present; otherwise growth can occur only in the presence of oxygen. Certain species, for example, when grown in a solution containing a nitrate, reduce this to a nitrite, evidently making use of the oxygen gained by this reduction. Others can grow as facultative anaërobes, provided suitable sugars are present. This may be illustrated by the growth of *Bacillus coli* in broth in a fermentation tube. In the absence of sugar, the growth is wholly confined to the open arm of the tube, that is, to aërobic conditions; in the presence of sugar, the growth is equally good in the closed arm.

Anaërobic bacteria are common in nature, though not as numerous as the aërobic forms. They find suitable conditions for growth in the soil and in decaying organic matter. Several species are known to produce disease in man and animals. Others are of importance in the canning industry, particularly in the canning of vegetables such as peas and corn. Yeasts are anaërobic or microaërophilic in the presence of suitable sugars. Most molds are aërobic, some are facultative, and very few are anaërobic.

It is evident that the different requirements with respect to oxygen must be taken into consideration in cultivation of bacteria. Many devices are in use to regulate oxygen supply. For a discussion of these methods a standard laboratory manual should be consulted.

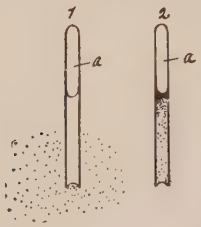


FIG. 104. — Chemotaxis. 1, a capillary tube containing an air bubble (*a*) and meat extract or peptone solution, thrust into a drop of water containing motile bacteria; 2, bacteria have entered the tube and are found in greatest numbers next the air bubble. (Adapted from Fischer.)

The Effect of Chemicals on Movement of Microörganisms. — Organisms that have the power of independent movement may have the direction of that motion determined in some measure by chemical substances. This phenomenon is termed *chemotaxis*. One of the most striking methods of demonstrating this phenomenon is that suggested by Fischer. A capillary tube having a very fine bore is sealed at one end and the other end thrust into a solution of peptone or beef extract. Capillarity will cause some of this liquid to enter the tube. The tip of this tube is then placed in a drop of water containing motile organisms. The direction of their motion may be watched under the microscope. At first they will be seen to be uniformly distributed throughout the drop. Soon, however, they will cluster about the end of the tube from which the peptone is diffusing. Before long practically all of the bacteria will be found at this point and within the tube itself. The cells apparently swim in the direction of the nutrient material (Fig. 104).

Substances which may serve as food for microörganisms do not always attract them. For example, cane sugar, which is an

excellent food for many forms, will not stimulate chemotaxis. It is probable in nature, however, that chemotaxis is of some use to organisms in finding food. The attraction of motile organisms by a chemical is termed *positive chemotaxis*. The reverse, or repulsion of organisms (negative chemotaxis), may be demonstrated by filling the capillary tube with alcohol or an acid. The organisms will be found to shun the vicinity of the tube. Another type of chemotaxis may be demonstrated by placing a drop of stagnant water containing a large number of motile organisms, both bacteria and protozoa, under a cover glass, being sure that several air bubbles are included also. In the course of a few minutes, the organisms will be found to have grouped themselves largely in concentric rings about these air bubbles (Fig. 105). It is evident that these organisms remain in the oxygen concentration which is most suited to their several requirements. This phenomenon is termed *aërotaxis*. Chemotaxis, as will be noted later, has an important part to play in the destruction of bacteria by the white blood corpuscles in the body. Bacteria which attract these blood cells are usually destroyed by them. Many bacteria secrete substances which are thus positively chemotactic.

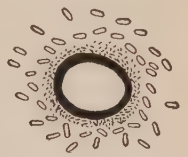


FIG. 105. — Aërotaxis. An air bubble under a cover glass surrounded by two rings of organisms, the inner bacterial, the outer protozoan. Each maintains itself in that oxygen concentration which is most suitable.

Effect of Chemicals on Direction of Growth. — The *direction of growth* of organisms not free to move may be influenced by chemicals. Such a determination of direction is called *chemotropism*. This may be of many types. The influence of moisture upon the direction of growth is of particular importance. This is termed *hydrotropism*. Molds growing upon the surface of nutrient media send their vegetative hyphæ into the substratum much as plant roots go down into the soil. The direction of growth in molds is not usually determined by gravity, for in general the mold hyphæ grow toward moisture. This may be termed *positive hydrotropism*. On the other hand, some spore-bearing organs of molds, such as conidiophores and sporangiophores, often show marked *negative hydrotropism*, the thread developing at right angles to a

moist surface, and producing its spores at some distance from such a surface. The importance of this fact has already been discussed under the heading of mold morphology. Most of the common mold genera, as Penicillium, Aspergillus, Mucor, and Rhizopus show this negative hydrotropism very distinctly.

Fig. 106. — Negative hydrotropism of mold sporangiophores. Rhizopus growing on the bottom and sides of a petri dish. Note that the sporangiophores arise in all instances at right angles to the surface from which they spring or bisect the angle made by these surfaces with each other.

The by-products of the growth of an organism may have a negative chemotropic influence upon other individuals of the same species or other threads of the same mold. An examination of a young culture of mold growing upon some solid medium, such as agar or gelatin in a petri dish, will frequently show the mold hyphæ radiating from the center in practically straight lines, the new branches rarely crossing the old. This is undoubtedly an example of negative chemotropism, each hyphal thread repelling others near it.

Effect of Chemicals upon Growth Rates. Stimulants and Nutrients. — The chemical constituents of the medium in which an organism is grown may stimulate the growth of microörganisms in any one or more of several ways.

The nutrient may be used more or less directly as a source of energy. A supply of energy is required by every cell; in part it is utilized in growth, in part it is released as heat, it is sometimes manifest in movement, occasionally in the production of light. This energy is secured in all cases by producing an oxidative change in some chemical compounds. For example, many species of yeast when grown in a medium containing dextrose permit it to diffuse into the cell where it is converted almost quantitatively into alcohol and carbon dioxide. These compounds are then excreted from the cell. In the transformation, energy is released to the cell. The production of alcohol and carbon dioxide from sugar by the yeast cell involves both oxidation and reduction. This process of securing growth energy is sometimes termed *dissimilation*, or catabolism.

Microörganisms which are most frequently studied in the

laboratory secure their growth energy by the oxidation of organic carbon compounds; there are, however, many organisms of considerable economic significance which secure growth energy by the oxidation of other substances, as hydrogen, sulphur, ammonia, nitrates, etc. These will be noted later.

The nutrient may be used more or less completely in building up cell parts, i.e., *it may be assimilated*. The chemical elements which are necessary for growth of microörganisms are in general the same as required by other plants and animals. Certainly here are to be included carbon, oxygen, hydrogen, nitrogen, sulphur, and phosphorus. To this list perhaps may be added iron, potassium, sodium, chlorine, calcium, and magnesium. These latter elements are doubtless *essential to growth*, but it has not been satisfactorily demonstrated in all cases that they are *essential cell parts*, in other words, that they are definitely assimilated.

Bacteria differ widely in respect to the type of chemical compounds in which the various chemical elements must be present in order to serve as nutrients. From the standpoint of their carbon needs two groups may readily be differentiated.

Most bacteria (particularly those most frequently studied in the laboratory) and practically all yeasts, molds and protozoa require organic carbon compounds. Some of these are *parasites* in that they live usually upon a living host, either animal or plant. They apparently require relatively complex food; when grown in the laboratory some will develop only when supplied with such substances as blood serum. When such parasitic organisms produce disease they are termed *pathogenic*. If they are not particularly detrimental, such as many of the bacteria living normally in the colon of man, they may be termed *commensals*. Organisms which require organic food but are not parasitic are frequently named *saprophytes* (if bacteria, yeasts or molds) or *saprozoites* (if protozoa). The saprophytic bacteria show marked differences in the types of organic compounds required. Particularly striking are the variations in the sources of the nitrogen. Some few forms (the *nitrogen-fixing* bacteria) are able to utilize atmospheric (free) nitrogen in their growth; they are quite important from the standpoint of soil fertility. Most bacteria, yeasts and molds can utilize

nitrogen from ammonia, a smaller number from nitrates. A few, particularly among the parasitic forms, apparently require the nitrogen to be present in organic compounds. This is probably true of most protozoa, indeed, of animals in general.

Some bacteria, probably among the most primitive forms of life, are able to utilize carbon dioxide or carbonates as nutrients. It is evident that these cannot be oxidized to secure energy. They are instead reduced and built up into organic compounds. Organisms which are thus capable of assimilating carbon dioxide are said to be *autotrophic* (sometimes *prototrophic*), that is, capable of manufacturing their own food. Energy is required by all autotrophic organisms thus to synthesize organic compounds. The autotrophic bacteria may be divided into two groups on the basis of the source of this energy. The first group is termed *photosynthetic* inasmuch as light energy is used for this purpose. Some of the order of sulphur bacteria (Thiobacteriales) possess a purple coloring material, *bacteriopurpurin*, which apparently functions for these bacteria in much the same fashion as does the pigment chlorophyll for the higher green plants. Such bacteria by absorbing certain of the sun's rays are able to build up organic carbon compounds from carbon dioxide in much the same manner as the green plant can utilize the energy of the sun's rays in the manufacture of starch. The second group of the autotrophic bacteria is said to be *chemosynthetic;* these organisms utilize energy secured by oxidation of various inorganic compounds for building organic compounds from carbon dioxide. Among the substances so utilized as energy sources are sulphur and its compounds, ammonia, nitrites, possibly iron and others. These autotrophic chemosynthetic microörganisms are to be regarded probably as among the most primitive of living things; they are able to grow in the absence of light, utilizing carbon dioxide and inorganic substances exclusively.

The substance may act as a growth stimulant without serving as an energy source or without being assimilated. Not infrequently chemicals may accelerate growth of microörganisms without being either directly assimilated and without serving as a direct source of growth energy. Sometimes such compounds absorb or remove

from the medium substances which are injurious to the growth. Such might be powdered charcoal. A chemical which acted as a buffer in preventing a rapid change in hydrogen ion concentration would also stimulate growth.

Inhibition of Growth and Destruction of Microörganisms by Chemicals.— Chemicals may not only stimulate the growth of an organism, they may partially or completely inhibit its growth, or they may destroy it. The following terms are commonly used with reference to the injurious effects of chemicals upon microorganisms.

A *germicide* is any substance which will kill microörganisms or germs. The term *antiseptic* in general is used to indicate any substance which will inhibit the growth of organisms without necessarily destroying them. A *preservative* is a substance which may be added to foods in order to inhibit the growth of microorganisms. Fundamentally the terms *antiseptic* and *preservative* therefore are synonymous. The former, however, is ordinarily used with reference to infection or to disease-producing organisms, and the latter with reference to organisms present in food and capable of causing decomposition and other undesirable changes. The term *disinfectant* is used to indicate a germicide especially efficient in the destruction of disease-producing organisms. All of these terms are frequently used rather loosely, sometimes even interchangeably. For example, it is very difficult to differentiate sharply between an antiseptic and a disinfectant. A weak solution of phenol will act as a preservative or antiseptic, while a stronger solution will destroy the bacteria present, acting as a disinfectant. The differences are therefore quantitative and not qualitative.

While an antiseptic may be defined as a substance which inhibits growth without destroying, it should be emphasized that cells which are not growing are dying, slowly perhaps. An antiseptic or preservative may therefore be defined as a substance which kills microorganisms relatively slowly, while a disinfectant is one that kills rapidly.

Characteristics of an Ideal Disinfectant. — Disinfectants and antiseptics are so frequently used and advocated that it is advisable to know something of their characteristics. This may be most

readily accomplished by noting certain of the characteristics which should be possessed by an ideal disinfectant. To the extent that a particular disinfectant measures up in its characteristics to those of the ideal disinfectant, it is valuable for general use. It is to be understood clearly, of course, that none of the disinfectants that have been described is ideal in all of its characteristics. The important characteristics of disinfectants are as follows:

1. *High Germicidal Power.* — The ideal disinfectant should possess high germicidal power. The ability of a particular disinfectant to kill microörganisms is usually compared with that of phenol. For the details of the methods of standardizing disinfectants the student should consult a standard laboratory guide. In making such comparisons it is customary to use the *Bacterium typhosum*, the cause of typhoid fever, as the test organism. A series of test tubes are prepared containing varying dilutions respectively of phenol and of the disinfectant to be tested. Equal numbers of *Bacterium typhosum* are then introduced into each tube. At intervals of two and one-half minutes samples are taken from each tube and transferred to a nutrient medium suitable for growth. The transfers are continued for a period of fifteen minutes. The concentration of phenol which is required to kill the organisms (so that no growth is secured when a loopful is transferred to sterile broth after two and one-half minutes exposure) is determined, likewise the concentration of the disinfectant being tested which will bring about the same results, that is, prove equally effective. The ratio between the dilutions of the disinfectant to be tested and the phenol is determined and the number recorded. The ratio between the concentrations of the phenol and of the test disinfectant required to kill in fifteen minutes is also determined. These two ratios are averaged and termed the *phenol coefficient* of the disinfectant under test. For example, if phenol kills in two and one-half minutes in a concentration of one to one hundred, and the disinfectant to be tested in one to five hundred, the first ratio would be five. If in fifteen minutes phenol kills in one to two hundred and the test disinfectant in one to twelve hundred, the ratio would be six. The average of the two would be five and one-half.

2. *Stability.* — The disinfectant to be most valuable should be relatively stable in the presence of organic matter. Some of the most powerful of the disinfectants combine with organic matter, forming insoluble compounds and pass out of solution almost completely. The strength of the disinfectant may be thereby rapidly decreased to a point where it no longer destroys micro-organisms. It should be noted that in some cases lack of stability may be desirable rather than undesirable. In certain instances it may be advantageous if the disinfectant is at first very active, and later loses its efficacy. For example, the water supplies of cities are often treated with gaseous chlorine. This is a powerful germicide. It rapidly combines with other compounds present in the water, and becomes powerless. The water may then be used for drinking; the chlorine taste will have disappeared. For all practical purposes, it first sterilizes the water and then disappears.

3. *Homogeneity.* — Disinfectants should be homogeneous in composition. Substances which may be bought in pure condition or in crystalline form such as mercuric chloride, are ideal from this point of view. Many of the commercial disinfectants, particularly those prepared from coal tars, may vary considerably in their composition from time to time, and consequently in their germicidal value.

4. *Solubility.* — The ideal disinfectant is one which will dissolve in all proportions in water. If it has a very high germicidal power, high solubility is not so important.

5. *Non-toxic to Higher Life.* — An ideal disinfectant would be one which is non-poisonous to man and animals. Obviously disinfectants which will kill one kind of cell and not injure another are difficult to find. Most of the valuable disinfectants are more or less injurious to tissues. Certain disinfectants, however, may be injected into the blood, exerting a more harmful influence upon microörganisms than upon tissues of the body, destroying the former without seriously injuring the latter. Such, for example, is the arsphenamine used in the treatment of syphilis.

6. *Non-corrosive and Non-bleaching.* — The ideal disinfectant will not corrode metals or bleach fabrics.

7. *Power of Penetration.* — A disinfectant should penetrate readily.

8. *Cheapness.* — An ideal disinfectant should be readily secured and relatively inexpensive.

9. *Deodorizing Power.* — A disinfectant is the more acceptable if it combines with or absorbs odors, *i.e.*, serves as a deodorant.

10. *Cleansing Power.* — The ability to remove dirt and grease is highly desirable.

How Germicides act. — Not all germicides destroy bacteria in the same manner. Several of the more important types of action will be noted.

Some disinfectants are such because of their powerful action as *oxidizing agents*. They destroy more or less completely the organic matter of the cell. For example, potassium permanganate acts in this manner. Other germicides cause more or less rapid digestion or *hydrolysis* of the cells, thus destroying them. A strong acid or a strong alkali may produce this change. Other chemicals apparently alter the *permeability* of the cell so that it cannot function. In other cases there is a direct *chemical union* with the cell, preceded in some cases by an adsorption of the germicide. The cell is killed as a result, the protoplasm in some cases is coagulated or precipitated. Such changes are produced by formaldehyde and by mercuric chloride. It is possible in other cases that the death of the cell is occasioned by a mechanical disruption or injury, such as a plasmoptysis or a plasmolysis (Fig. 107). This type of action is the probable explanation of the preservative action of strong brines and syrups. In some cases a chemical may prove injurious to the cells of microörganisms because primarily it accelerates or stimulates the activity of some other substance. The addition of common salt, for example, to a solution increases the germicidal activity of phenol.

Some disinfectants, particularly the salts of the heavy metals, are most efficient in solutions in which they are strongly ionized. For example, mercuric chloride dissolved in absolute alcohol is far less efficient than when in solution in water. It is the mercury ion which is poisonous; the compound does not ionize in alcohol but does in water, hence the greater efficiency of the latter solution. Other

substances present may markedly modify the action of the disinfectant. The amount of ionization, for example, may be wholly changed by the addition of salts.

Disinfectants, antiseptics, and preservatives in common use may, for convenience, be grouped under the following heads: salts of the heavy metals, the halogens and their compounds, alkalies and mineral acids, and organic compounds and dyes.

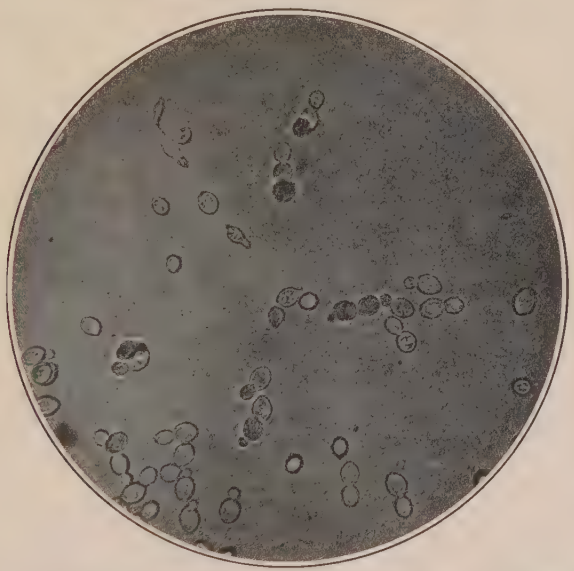


FIG. 107. — Torulæ from pickle brine. These organisms can develop in relatively high concentrations of common salt. (× 500.)

Salts of the Heavy Metals. — The soluble salts of the heavy metals — mercury, silver, copper, and iron — are more or less efficient as disinfectants.

Mercuric chloride has been frequently used as a disinfectant, and under the right conditions it is efficient. It combines with protoplasm to form an albuminate of mercury, an insoluble compound. It must be used in considerable excess when disinfection of solutions containing quantities of organic matter is desired (feces, for example), because it forms insoluble compounds with many of these

as well. It is apt to form an impervious coating over the surface of solid particles and protect the bacteria in the interior from injury. Mercuric chloride in high dilutions acts as an antiseptic, preventing growth of organisms in a solution containing one part in 100,000 or even 1,000,000. It is usually employed as a disinfectant in a strength of 1–500 or 1–1000. Spores of most bacteria are killed in water by this concentration in an hour or less; vegetative cells are destroyed within a few minutes. This compound may be purchased mixed with other substances in tablet form, the other ingredients being such as lessen the formation of insoluble precipitates, thus rendering the solution more effective. Mercuric chloride is so toxic to animals and man, however, that its use in the household is not advisable. It also corrodes metals with which its solutions come into contact. *Mercuric iodide* may be used as is the chloride and is even more efficient.

Silver nitrate is relatively efficient as a disinfectant, and is used in medicine, particularly to kill bacteria on body surfaces. Colloidal suspensions of silver are also used on mucous membranes.

Copper is one of the most efficient of chemicals in the elimination of algæ from water reservoirs. Such filamentous forms as Spirogyra are killed by even as low a concentration as 1 part in 1,000,000. It is usually used in the form of copper sulphate. It has been added to contaminated water in supplies in cities to destroy pathogenic bacteria. For this purpose, however, it has been superseded in most instances by calcium hypochlorite. It has proved useful in some cases in the sanitary control of swimming pools. It is a valuable fungicide and in combination with lime (as Bordeaux mixtures) is commonly used in sprays to prevent plant diseases.

Iron salts, particularly the sulphate, were formerly employed to a considerable extent in disinfection, but are much less efficient than was once supposed, and are now little used.

Halogens. — All of the halogens (chlorine, bromine, iodine, fluorine) are active disinfectants in a free state, and many of their compounds are used as antiseptics and preservatives.

Chlorine, free or uncombined, is one of the most widely used of disinfectants. It is added to the water supply in many cities when

there is danger of pollution. As was noted previously, it has the advantage of being very active as a disinfectant, but it soon disappears as free chlorine, combining with other substances present. It is used in concentrations of one-half or less to several parts per million. It has also found extensive use in the disinfection of water in swimming pools. It has also been used to mix with sewage effluents for the purpose of sterilization. The *hypochlorites*, calcium hypochlorite in particular, have also been utilized to prevent infection through contaminated city water supplies. A small proportion of this material added to water before it is pumped through the mains will effectually rid it of any disease-producing organisms that may be present. In the amounts used it apparently does not materially injure the water for domestic purposes. An alkaline solution of hypochlorite has been extensively used in wound disinfections in medicine; it has been named Dakin solution, after the man who developed it. A strong alkaline solution of hypochlorite under the trade name of antiformin has been widely used as a disinfectant. *Chlorides* are not generally efficient in low concentration, but when present in excess exert a decided preservative action. Few organisms can develop in a brine containing more than 20 per cent of common salt. Advantage is taken of this fact in the use of salt and brine in pickling and preserving foods.

Fluorine, as sodium fluoride, is sometimes used to arrest the growth of organisms when it is desired to study the activity of enzymes present. Most enzymes act readily even in a one per cent solution of sodium fluoride, while the organisms present are inhibited in growth or completely destroyed.

Iodine is most commonly employed in solution in alcohol. It is a reasonably satisfactory disinfectant.

Alkalies and Inorganic Acids. — Strong alkalies dissolve the protein contents of cells and thus destroy them. Strong acids act in a similar manner. Dilute solutions of acids and alkalies prevent multiplication of organisms by rendering the reaction of the medium inimical to growth.

Lime, either calcium oxide or hydroxide, is frequently used as a disinfectant in privy vaults, and in the form of whitewash in out-

buildings. It is efficient when fresh, but on exposure to the air is gradually changed into calcium carbonate which has little disinfecting value. Whitewash is an excellent aid to the preservation of cleanliness, but it should not be relied upon as an infallible disinfectant.

Alkaline compounds such as sodium carbonate, sodium hydroxide, and sodium phosphate are the principal constituents of the washing powders commonly employed in creameries and other establishments where many bottles are washed. If sufficiently concentrated, these are effective disinfectants as well as detergents.

Sulphurous acid is one of the most commonly used of disinfectants, particularly in fumigation. The sulphur dioxide is prepared by burning sulphur. When dry, it is of little value, but in the presence of moisture, as in vapor-saturated air, it is quite efficient. Under these conditions sulphurous acid is formed. It has the disadvantage of being an active bleaching agent. It will remove the color from carpets, curtains, wall paper, etc., when moist. It is still commonly used in the fumigation of ship holds, where it is desired not only to destroy microörganisms of all kinds, but rats, insects, and other vermin as well. About four pounds of sulphur should be burned to every thousand cubic feet of air space. Water should be boiled or steam should be let into the room at the same time. As a household fumigant it has been almost entirely replaced by formaldehyde.

Organic Compounds. — The most commonly used organic disinfectants, antiseptics and preservatives are formaldehyde, chloroform, iodoform, alcohol, organic acids such as acetic and lactic, phenol and its derivatives, the cresols, benzoic acid, salicylic acid, certain of the aniline dyes, toluol, and a few of the essential oils such as oils of peppermint, clove, thyme, and cinnamon.

Formaldehyde. — Formaldehyde (HCHO) is a gas readily soluble in water. It is commonly sold as formalin, a 40 per cent or saturated solution of formaldehyde in water. It may also be secured in the form of one of its polymers called "paraformaldehyde" and "trioxymethylene." Both of these polymers are readily converted into formaldehyde by heat. Formaldehyde may be prepared also by partial oxidation of methyl alcohol which occurs when it is

passed through finely divided platinum raised to red heat. Formaldehyde is at present more commonly used in fumigation than any other gas. It does not injure the texture of fabrics and very rarely modifies or changes their colors in the least. It can therefore be used for the disinfection of furnished rooms. It is active only in the presence of moisture, and during fumigation a room should have its atmosphere as nearly saturated as practicable. Formaldehyde destroys organisms by uniting chemically with the protoplasm. About one per cent by volume of the gas is necessary for efficient disinfection of rooms. Many methods have been devised for the production of formaldehyde in quantities sufficient for fumigation. The most common method used is that of heating formalin directly over a flame in a suitable vessel. The heat at first converts most of the formaldehyde into paraformaldehyde, but as the water evaporates this is broken up and given off as formaldehyde gas. By this method a sufficient quantity of moisture is always certainly introduced. Much the same result can be obtained by placing potassium permanganate crystals in an earthen or wooden vessel and pouring the solution of formalin over them. The rapid oxidation of a part of the formaldehyde by the permanganate results in the production of a sufficient amount of heat to vaporize most of the remainder. This method may be used conveniently where it would be unwise to introduce a fire into a closed room. Many types of apparatus are also on the market for the production of formaldehyde from its isomers. In addition to the use of formaldehyde as a fumigant, it is sometimes utilized as a preservative in food, as in milk. Its use for this purpose is now generally forbidden by law.

Chloroform ($CHCl_3$) when pure does not readily destroy microorganisms. It is most important as an antiseptic. It may be added to certain materials such as blood serum and will prevent or retard the growth of bacteria. It is also used as an antiseptic in the investigation of changes brought about by enzymes in order to prevent the growth of organisms that might obscure or modify the results of enzyme action. Within recent years it has been much less used than formerly, inasmuch as it has been found that some organisms can grow readily in media lying above chloroform;

in a tube of bouillon, for example, having a layer of chloroform in the bottom.

Iodoform (CHI_3) is very commonly used as an antiseptic by physicians. It has but little direct action upon microörganisms and is not itself actively an antiseptic or disinfectant. When mixed with organic compounds, however, it is decomposed to a certain degree and the iodine is freed. This has a distinct disinfecting action.

Certain of the *organic acids*, particularly *lactic* ($CH_3.CHOH.COOH$) and *acetic* ($CH_3.COOH$) are among the most commonly used preservatives. The decomposition of milk is prevented by the formation of lactic acid by certain bacteria, and it is only after this acid has been oxidized to carbon dioxide and water by certain molds that further changes in the milk take place and the protein compounds are broken down. Lactic acid is also formed in sauerkraut and various types of pickles (such as dill pickles) by the fermentation of the sugars present. It accumulates in sufficient quantities to inhibit more or less completely the growth of putrefactive organisms. Acetic acid, the active constituent of vinegar, is formed usually by the oxidation of alcohols. It inhibits the growth of many microörganisms and is frequently used as a preservative, as in pickled meats, vegetables, and fruits.

Alcohol unmixed with water has very little disinfecting value. Seventy per cent solutions are most efficient.

Phenol (C_6H_5OH) or carbolic acid is one of the most commonly used and most valuable of the disinfectants. In the proportion of one part in a thousand, it is an antiseptic. It is commonly used in 5 per cent solution, which will destroy non-spore-bearing microörganisms very quickly. Some bacterial spores, however, can resist its action for a considerable time. The addition of 0.5 per cent of hydrochloric acid increases its effectiveness considerably. All of the common pathogenic bacteria, with the exception of those producing spores, are destroyed within a few minutes by such a mixture. It is particularly valuable because it does not form insoluble precipitates with albuminous substances. It is the *standard* disinfectant, that is, it is the compound with which other germicides are compared in determining their disin-

fecting value. It should not be inferred from this fact that it is an ideal, or even the best, disinfectant.

Cresol or *methyl phenol* ($CH_3.C_6H_4.OH$) is one of the chief constituents of many of the proprietary disinfecting compounds upon the market. Three types are known to the chemist, the meta-, the ortho-, and the paracresol. A mixture of these three is called tricresol. A saturated solution in water contains about $2\frac{1}{2}$ per cent. In this proportion it is two or three times as efficient as phenol. The cresols are sometimes mixed with strong acids or alkalies to increase their solubility. Such mixtures are sold under various trade names and in most cases are efficient and reliable.

Benzoic acid ($C_6H_5.COOH$) and *sodium benzoate* ($C_6H_5.COONa$), *salicylic acid* ($C_6H_4.OH.COOH$) and *sodium salicylate* ($C_6H_4.OH.COONa$) are commonly used as preservatives in foods, usually in quantities of 0.2 per cent or less. In the United States the use of salicylic acid or salicylates in food is usually forbidden, while the use of benzoate is permitted, provided its presence and amount is stated.

Miscellaneous Antiseptics. — Essential oils such as *thymol* and *eucalyptol* exert a marked antiseptic or even disinfectant action. Others, such as oils of peppermint, cloves, cinnamon, etc., are somewhat less effective. The addition of spices and their essential oils undoubtedly plays a part in the preservation of certain food products.

Hydrogen peroxide (H_2O_2) will inhibit bacterial growth in dilutions as high as 1–20,000. Its action is probably similar to ozone (O_3).

Potassium permanganate is an active oxidizing agent in solution, destroying most organic matter with which it comes in contact.

Boric acid is sometimes used in saturated solution as an antiseptic, particularly in treatment of bacterial infections of mucous surfaces.

Effects of One Organism upon Another. Symbiosis, Antibiosis, Metabiosis, and Commensalism. — The substances excreted by microörganisms and the compounds they form are often of significance to other organisms. The relationships are denoted by the terms symbiosis, antibiosis, metabiosis, and commensalism.

There are many examples of these relationships. Sometimes bacteria or molds grow in a symbiotic relationship to higher plants. For example, in the nodules on the roots of leguminous plants, such as the pea and bean, the bacteria take up nitrogen from the air and this nitrogen becomes available in part at least for the use of the higher plant. The bacteria on the other hand secure their carbonaceous food from the roots. Such a symbiotic relationship is evidently mutually beneficial. In other cases the relationship is between microörganisms and animals. Many insects, for example, contain certain organs (called *mycetocytes* and *mycetomata*) which always harbor certain species of microörganisms. *Antibiosis* is the reverse of the preceding; it is the condition that obtains when organisms are inimical to each other's growth. The lactic acid bacteria in milk tend to prevent the growth of putrefactive organisms; the thickly crowded colonies on a heavily seeded plate are always smaller than on one in which fewer appear and many are totally inhibited. *Metabiosis* is that condition which exists when one organism paves the way for the growth of another. Yeast by its production of alcohol in a sugar solution prepares the medium for the growth of the acetic bacteria that oxidize the alcohol to acetic acid and water.

An organism is said to be a *commensal* when it lives on the waste products of another without injuring it. The bacteria in the intestines of man and animals are largely of this nature. The differentiation of parasites into commensals and pathogens has previously been noted.

SECTION IV

EFFECT OF ORGANISMS ON THEIR ENVIRONMENT. INCLUDING FERMENTATIONS

CHAPTER XIX

PHYSICAL EFFECTS PRODUCED BY MICROÖRGANISMS

Microörganisms may produce physical changes in their environment in several ways. They may develop heat and raise the temperature, in some cases they may produce light, they may alter the osmotic pressure or the viscosity of the medium in which they grow.

Heat Production by Microörganisms. — All organisms liberate more or less energy in the form of heat as a result of the oxidation processes. The amount of heat produced by some organisms is much greater than that evolved by others. Organisms producing large quantities of heat are said to be *thermogenic*. Such bacteria are usually aërobic. They are widely distributed in nature. They are in evidence in every decaying heap of straw and manure. The heating of a manure pile is due to the presence of optimum conditions for growth of this type of organisms. Temperatures as high as 70° or 80° C. may be reached. Frequently the ensilage in a silo undergoes such a process of heating. It seems evident from the results of recent investigations that in well-packed ensilage high temperatures are not usually reached except within a few feet of the surface or at points where oxygen has some access. In the heating of products such as ensilage, we have to do with other factors in addition to bacteria, inasmuch as there are oxidizing enzymes present in the living plant cells, and these may remain active for a time and start the action. The heat produced in the decay of manure is utilized by the gardener in the preparation of hotbeds. A layer of manure covered with soil inclosed in glass frames will maintain the temperature of the soil and air high enough to prevent damage from frost and to force young plants into rapid growth.

There is evidence that in masses of damp straw or hay the temperature may increase to a point that spontaneous oxidative

changes occur, and the temperature continues to rise until " spontaneous combustion " occurs.

Light Production. — Organisms which are phosphorescent or capable of producing light are said to be *photogenic*. Many species of such have been described. Most of them have as their natural habitat the ocean. It is not difficult to cultivate these organisms

FIG. 108. — Photogenic bacteria colonies on a plate photographed by means of their own light. (Lafar.)

upon artificial media containing preferably a considerable proportion of common salt and sugar. Under these conditions a slant agar culture in a test tube will give off sufficient light so that the time of day may be told by holding it against the face of a watch when in a dark room. These organisms are sometimes observed in fish markets or upon decaying fish upon the ocean beach, causing the fish at night to glow with a phosphorescent light. They probably account in part at times for the phosphorescence of salt water. Some have been identified from the luminescent organs of certain fish and cephalopods. None of them, however, is of great economic

importance. The conditions for development are plenty of oxygen and food material, usually the presence of common salt, and a moderate temperature. In addition to the bacteria, many species of fungi are phosphorescent. The mycelium of some of the Basidiomycetes (toadstools and mushrooms) is the common cause of phosphorescence in decaying wood and leaves. Certain of the Hyphomycetes or molds are also known to possess this faculty. It is probable that this phosphorescence is to be explained as the result of the reaction of certain waste products or by-products, for these organisms can live and grow luxuriantly under some conditions without producing light. Under other conditions where the growth is equally rapid, they are markedly phosphorescent.

Changes in the Consistency of the Medium. — The changes brought about by microörganisms in the consistency of the medium in which they grow may be divided into two types, those resulting from analytic and those resulting from synthetic changes.

Changes due to Analytic Processes. — Many microörganisms are capable of digesting, that is, rendering soluble, solid materials or solid organic substances which may be useful to them as food. The insoluble carbohydrates, such as cellulose and starch, are hydrolyzed and converted into the simplest sugars. A starch suspension or solution, for example, inoculated with an amylolytic organism is rapidly changed from a thick, viscous solution to one which is relatively clear and limpid. Similar changes are brought about in insoluble proteins and other nitrogenous compounds. Certain organisms when inoculated into a medium solidified by the use of gelatin, hydrolyze this gelatin, thus destroying its gelatinizing power. Flesh and boiled white of egg or similar substances when acted upon by certain bacteria are rapidly converted into soluble substances, or digested. From the standpoint of the organism, this is an important characteristic, inasmuch as it puts insoluble potential food materials into solution and usually in such form that diffusion may take place through the cell wall and plasma membrane, and the cells of the organism may utilize the material as food.

Changes due to Synthetic Processes. — Certain organisms may change the consistency of a medium to a considerable degree by

products of synthetic action. This is particularly true of those forms which are capable of producing slimes and gums in liquid media. These are usually the result of the secretion of an enormously thickened cell wall or capsule, which rapidly swells and goes into a state of semi-solution. It is easy to observe the various stages in the production of these materials by the examination of the organisms under the microscope. These slimes or gums may be nitrogenous and mucinlike, or they may be polysaccharides such as dextrans, mannans, etc. These organisms are of considerable economic importance as they are responsible for the production of slimy milk, slimy bread, and gums and slimes in sugar manufacture. In recent years it has been argued by certain bacteriologists that many of the so-called vegetable gums produced by higher plants, such as the acacia and astragalus, are due primarily not to direct production by the plant itself, but to the growth of certain microörganisms in the sap exuded.

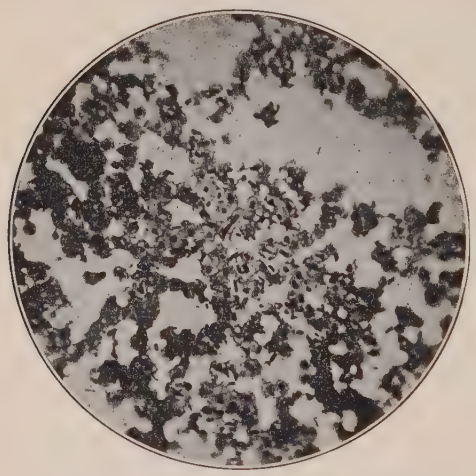


Fig. 109. — Microörganisms causing slimy milk. Note the presence of capsules, and that the capsular material in many places shows evidences of partial solution in the liquid. (Photomicrograph, × 1000.)

Changes in Osmotic Pressure. — The osmotic pressure of any substance in solution is proportional to the number of molecules (or of molecules and ions) in a given volume of the solution. It is evident, therefore, that any changes in a compound in solution which will increase the number of molecules to a given volume will increase at the same time the osmotic pressure. This may be illustrated by the change which occurs in the digestion or saccharification of starch. Starch when boiled passes into a colloidal

solution usually somewhat viscous. If this is inoculated with some organism capable of digesting it, the starch molecules are rapidly broken down into simpler substances, at first into the polysaccharides known as dextrins. The starch in solution has very little osmotic pressure. The breaking down of the large starch molecule into simpler dextrin molecules increases the pressure considerably. When these are finally broken down into maltose or malt sugar, the osmotic pressure is greatly increased and when, as may occur, the maltose is broken down into dextrose, the osmotic pressure is again much increased. It is evident, therefore, that the analytic action of organisms upon both insoluble and soluble organic molecules is in general to increase the osmotic pressure, while the reverse or synthetic action is to decrease the osmotic pressure. These facts are of some importance in explaining certain changes which occur in the tissues of man and animals in certain types of disease. When microörganisms gain entrance to a tissue, destroy the cells and break them down into simpler soluble and crystalloidal substances, they greatly increase the concentration of solutes and tend to draw the water from the adjoining blood vessels and tissues to them.

CHAPTER XX

CHEMICAL CHANGES PRODUCED BY ORGANISMS

Cycles of the Elements

The specific changes brought about in particular compounds will be discussed in greater detail in the succeeding chapters. It is the purpose of this chapter to outline briefly the nature of certain of the changes induced, with particular reference to the so-called cycles of some of the elements, and to note the part played by microörganisms in inducing these changes. The most important elements to be considered are nitrogen, carbon, sulphur, and phosphorus.

The Cycle of Nitrogen in Nature. — Nitrogen exists in nature in three principal forms, free in the air as a gas, in inorganic compounds such as ammonia, nitrites, and nitrates, and in organic compounds such as amino-acids and proteins. Microörganisms are important in changing nitrogen from one form or combination to another; in fact, without their activity in this matter it would soon become impossible for higher animals and plants to exist on the earth.

It is most convenient to start with complex organic nitrogenous compounds in a study of the nitrogen cycle. The changes to be considered are ammonification, nitrification, nitrogen assimilation, denitrification, and nitrogen fixation.

Ammonification. — The conversion of complex nitrogenous compounds into simpler forms, and ultimately into ammonia, is termed ammonification. This occurs in several distinct stages. The complex protein molecule is broken up into somewhat simpler compounds, the proteoses, and then into peptones; this process may be termed *peptonization.* These peptones are broken down by various organisms with the formation of polypeptids and amino-

acids primarily and a considerable number of secondary products. The amino-acids are still further decomposed with the production of ammonia. This whole process of successive cleavages of proteins is sometimes termed *proteolysis*. One of the principal nitrogenous waste products of the dissimilation of proteins in the animal body is urea. This is actively transformed by certain bacteria into ammonium carbonate. It is evident that all organic nitrogenous compounds are ultimately reduced to ammonia by the process of *ammonification*. This is of very great economic importance in agriculture, as nitrogen in this form is readily changed so as to become available to higher plants.

The various steps in ammonification may be brought about by different organisms. A few species can attack native proteins; many however can utilize and change only the peptones, the peptids, and amino-acids. These will be considered in some detail later. The organisms changing urea to ammonium carbonate constitute a very distinct group.

Nitrification. — Certain bacteria, particularly a coccus called *Nitrosomonas*, are able to oxidize ammonia to nitrous acid. They are common in the soil. They secure their energy for growth by this change. The nitrous acid is normally at once neutralized by the bases of the soil thus forming nitrites. These nitrites soon undergo the next change, oxidation to *nitrates* by other species of soil bacteria. The nitrates, and to a less degree the ammonia, constitute the sources of nitrogen for higher plants. No nitrogenous manure is effective in increasing crop yield that is not capable of being ammonified and nitrified.

Nitrogen Assimilation. — The discussion of the nitrogen cycle is not complete without a consideration of the nitrogen assimilation, even though this is in large part a function of the higher plants. The nitrates, and to a less degree the ammonia, produced by bacterial activity in the soil are taken up through the roots and built up into protoplasm and complex proteins. These may decay or they may be eaten by animals, but ultimately they are decomposed by microörganisms. This alternation of synthesis of proteins by higher plants and disintegration by micröorganisms constitutes the principal part of the nitrogen cycle.

Denitrification. — Nitrates in the absence of oxygen and in the presence of organic matter may be reduced to nitrites by bacterial activity, and these nitrites further decomposed with evolution of free nitrogen. Under these conditions microörganisms take the oxygen from the molecule of nitrate or nitrite. Some species, for example, will live under anaërobic conditions, if nitrates are present, otherwise they are aërobic. This fact is of some significance in agriculture in explaining loss of fertility in water-logged soils.

Nitrogen Fixation. — It is evident that if gaseous nitrogen is lost from the cycle as a result of denitrification, there must be some method whereby it can be again fixed or combined. A considerable number of species among the bacteria and molds are known to possess this power.

Certain of the higher plants have moldlike fungi which live upon their roots and which in some cases probably take up the atmospheric nitrogen. Among these are the alders, Russian olives, and certain other trees, the orchids and many plants living in peat bogs and swamps. These symbiotic combinations of roots and organisms are termed *mycorrhizas*.

Various species belonging to the bacterial genus Rhizobium (as *Rhizobium leguminosarum*) produce nodules or tubercles on the roots of many leguminous plants such as the bean, pea, clover, and alfalfa. These swellings are found to be made up of cells tightly packed with bacteria. These organisms take nitrogen from the air and directly or indirectly transfer it in part to the host plant. Legumes, unlike most other plants, therefore, can grow in soil devoid of nitrogen, provided the roots are supplied with nodules. The efficiency of legumes in increasing the fertility of the soil is due to this fixation of nitrogen.

There are also free-living soil bacteria which can take up nitrogen from the air. These belong to two groups, anaërobes and aërobes. Some spore-bearing soil bacilli (clostridia) in the presence of proper food, such as certain carbohydrates, can fix some nitrogen under anaërobic conditions. These forms are probably not very important in the soil. Much more important are the aërobic organisms called *Azotobacter*. These secure energy for the fixation of

nitrogen by the oxidation of carbohydrates. They are probably of considerable significance in soil fertility.

Summary. — These changes of nitrogen can best be summarized by use of the accompanying diagram (Fig. 110). The direction

FIG. 110. — The Nitrogen Cycle. Changes brought about by bacteria are represented by solid lines, other changes by broken lines.

of changes is indicated by arrows, the changes produced by microorganisms are indicated by solid lines, other changes by broken lines.

The student should note that an understanding of the nitrogen cycle and of the various changes involved is very useful as a basis for understanding many of the changes of economic significance produced by bacteria. A study of soil bacteriology consists in large part of a consideration of the changes produced in nitrogenous compounds by microörganisms. The ripening of certain foods includes consideration of proteolysis and ammonification; food spoilage is frequently concerned with changes in proteins and their products of hydrolysis. In problems of sewage disposal and water purification the processes of ammonification and nitrification are of great practical significance.

Carbon Cycle. — The cycle of carbon in nature as affected by micoörganisms is somewhat simpler than that of nitrogen. It may be best understood by emphasizing two fundamental facts, first that all living organisms, plants and animals alike, are continuously developing carbon dioxide; second, that some plants can synthesize organic compounds, principally carbohydrates and fats,

from carbon dioxide. The conditions for these antithetic transformations should be understood. All active cells are constantly breaking down carbon compounds; some can also build them up. All plants containing chlorophyll, or leaf green, use carbon dioxide and water to produce starch and sugars, gaining the energy necessary by means of the absorption of sunlight. A few bacteria containing bacteriopurpurin are also capable of using light for this purpose. Some forms oxidize ammonia to nitrites, nitrites to nitrates, hydrogen sulphide to sulphur or sulphur to sulphuric acid and utilize the energy thus secured in building up food materials.

It is evident that the carbon cycle consists of the alternate building up of carbon into organic compounds and their subsequent disintegration with ultimate oxidation of the carbon to carbon dioxide (Fig. 111). The various stages in decomposition and the

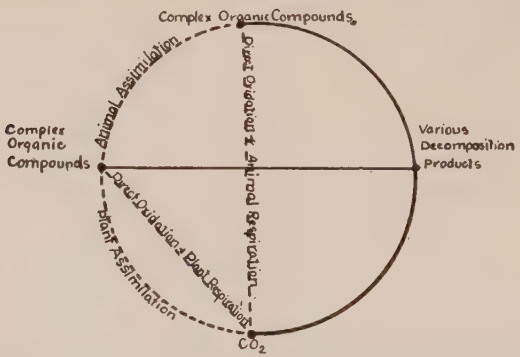


FIG. 111. — Carbon Cycle. Changes brought about by microörganisms are represented by solid lines, other changes by broken lines.

products of action of organisms on carbon compounds will be considered in a subsequent chapter. Many of these changes are of great economic importance.

Sulphur Cycle. — The decomposition of organic compounds containing sulphur usually results in the evolution of hydrogen sulphide. This compound is readily oxidized by many aërobic bacteria with the production of free sulphur and of sulphuric acid. These organisms are abundant in sewage and in the water of sulphur springs. In the latter they may form masses of a very consider-

able size. The sulphur granules may be seen within the cells of the organisms on microscopic examination. On the other hand, reduction of sulphur compounds with formation of hydrogen sulphide occurs when sulphates in the presence of organic matter are subjected to anaërobic conditions. The sewages of some cities, for example, are particularly offensive because the city water supply contains sulphates in considerable quantity, the bacteria causing decomposition of the organic matter of the sewage reduce the sulphates (probably for the oxygen they contain) and the sulphide is formed. The economic significance of certain of these changes will be noted later.

Other Elemental Cycles. — Somewhat similar cycles of elements in nature showing the influence of microörganisms upon the changes may be established, notably for phosphorus, potassium, and calcium.

CHAPTER XXI

GENERAL DISCUSSION OF FERMENTATION

The term *fermentation* comes from the Latin root meaning *to boil*. The type of fermentation familiar to the ancients, and one of the first studied, was that of the conversion of sugar into alcohol and carbon dioxide. Fermenting saccharine solutions always show a large number of gas bubbles rising to the top much as the bubbles of steam rise in boiling water, hence the name fermentation. Its meaning has been much extended in modern times. It has been variously defined; sometimes to include all changes brought about in carbohydrates by the action of organisms, at other times to include changes of all kinds brought about by organisms not only in carbohydrates but also in proteins, fats, and other substances. The term as used in this volume will include all chemical changes brought about by microörganisms directly or indirectly.

The word *putrefaction* has likewise been variously defined. It is commonly understood to indicate decomposition of organic substances, particularly nitrogenous materials, under anaërobic conditions with the development of unpleasant odors.

Decay is defined as decomposition of organic matter brought about in the presence of air and without the development of unpleasant odors. In practice, it is very difficult to differentiate between putrefaction and decay. In fact, the distinction is probably largely quantitative and not qualitative.

Zymotechnique is that branch of applied science which treats of fermentations, particularly those that are of economic importance.

Ferments. — A great advance was made in the science of microbiology when it was definitely proved by Pasteur and others that organisms such as yeasts are capable of setting up fermentations and transforming organic compounds. At the time of the promulgation of this theory it was believed by chemists that any organisms

which might be found in fermenting liquids were wholly incidental and not the cause of the fermentation, which they held to be strictly chemical in nature. The proof that living organisms do cause fermentations induced biologists to classify ferments (that is, anything capable of inciting fermentation) into two groups, organized and unorganized. The *organized ferment* was defined as a living cell which produced fermentation. The *unorganized ferment* was defined as a product of cell growth and activity capable of bringing about fermentation. In the first group (or organized ferments) were included the yeasts producing alcoholic fermentations, the lactic acid bacteria, acetic acid bacteria, etc., while in the second group (or unorganized ferments) were included the digestive enzymes such as the pepsin and rennin of the stomach. These unorganized ferments are generally termed *enzymes*. More recently we have come to believe that all fermentations brought about directly or indirectly by living cells are in fact produced by enzymes. In some cases the enzymes are liberated from the cell and produce their changes in the medium surrounding the organism. In other cases the enzyme remains in the cell and is more or less intimately bound up with the protoplasm of the cell. The terms organized and unorganized, therefore, have lost their original significance, and one now speaks of changes as brought about by *extracellular* and *intracellular enzymes*.

Origin of the Products of Fermentation. — There are five relatively distinct methods whereby microörganisms can bring about chemical changes or fermentations. Not all of them are significant in each case.

Analytic Action of Extracellular Enzymes. — Many organisms excrete enzymes which can bring about analytic changes in organic, sometimes in inorganic, substances outside of the cell. These changes may be of many types. Some extracellular enzymes digest starch, breaking it down into sugars, or digest insoluble proteins, breaking them down into proteoses and peptones. These enzymes are doubtless useful to the organism because they render certain insoluble food materials soluble and diffusible. Once in solution certain of these materials may pass through the cell wall and ectoplast of the organism and be utilized within the proto-

plasm as food. An essentially similar action is the breaking down of colloidal substances into simpler materials. The soluble proteins, for example, will not diffuse through plant membranes, and cannot be utilized directly by the cell of the microörganism as food. Certain of the extracellular enzymes break these down into crystalloidal substances, which are readily diffusible.

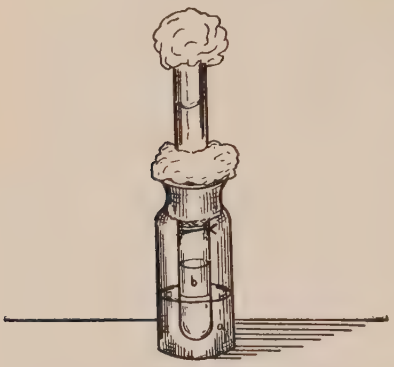


FIG. 112. — Kellerman's apparatus for demonstrating extracellular enzymes. *a*, nutrient gelatine; *b*, Celloidon sac filled with broth inoculated with organism to be tested. The production of a gelatinase is manifested by the liquefaction of the gelatin surrounding the sac.

In the discussion of the osmotic relationships of microorganisms it was noted that the ectoplast or outer layer of the protoplasm acted as a semi-permeable membrane; that is, certain substances in solution could pass through while others would be held back. In other words, it is not only necessary that a food substance be in solution, but it must also be of such a nature that it can diffuse through the cell wall and ectoplast of the organism before it can be utilized as food. For example, cane sugar ($C_{12}H_{22}O_{11}$) will not pass through most plant membranes, while dextrose and levulose ($C_6H_{12}O_6$) are readily diffusible. Organisms living in solutions containing cane sugar, therefore, generally "invert" (hydrolyze) the sugar by transforming it into a mixture of dextrose and levulose which can pass into the cell and be utilized. The analytic action of extracellular enzymes may therefore be summarized as digestive in that they render soluble certain food substances, transform colloids into crystalloids, and change substances which do not penetrate the ectoplast into those which do.

Analytic Action of Intracellular Enzymes. — Any living cell, whether plant or animal, possesses intracellular enzymes, inasmuch as these are essential to the life processes of the cell. The products of the analytic action of these intracellular enzymes

are therefore constantly being given off by all active cells. The amount of these materials depends very largely upon the mode of life adopted by the organism. Those organisms, for example, that live under anaërobic conditions and are dependent for their energy supply upon the destruction of large quantities of organic matter bring about these changes in the cell and excrete the waste products in large quantities. Many of the economic fermentations of importance are of this character, *e.g.*, the conversion of sugar into alcohol and carbon dioxide, and the production of lactic and butyric acids. Some intracellular enzymes, however, are very active under aërobic conditions. Such for example is the enzyme of the mother of vinegar, which converts alcohol into acetic acid.

Analytic Action of Autolytic Enzymes. — All cells contain enzymes which are capable of bringing about a more or less complete digestion of the cell protoplasm upon the death of the cell. When large numbers of organisms have grown to maturity in a nutrient solution, a considerable proportion of these are destroyed and their bodies undergo partial digestion or *autolysis*. In consequence the nitrogen constituents in the body of the organism go into solution. This is of importance in the preparation of certain vaccines and materials for treatment in preventing disease. It is found that the products of autolytic digestion of certain of these disease-producing organisms are poisonous.

Synthetic Action of Organisms. — In a previous chapter the methods of intracellular and extracellular syntheses due to microorganisms have been discussed. The products of these syntheses may be of importance in fermentation. Enzymes are produced intracellularly, as are also certain toxins or poisonous materials. The gums and mucins are largely extracellular in origin.

Secondary Products. — The four sources outlined above constitute the primary sources of the products of action of microorganisms. Certain products may be regarded as secondary. The production of acids, for example, in a solution may bring about changes in other substances present. The presence of carbon dioxide in the soil gradually brings into solution the relatively insoluble compounds of potassium and phosphorus. Acids may also injure or corrode metal vessels in which food products are stored.

CHAPTER XXII

ENZYMES OF MICROÖRGANISMS AND THEIR ACTIVITIES

Definitions. — *Enzymes* may be defined as *organic catalysts* produced by the cells of plants and animals. They are capable of bringing about changes in various substances without becoming a part of the final product of the action, and are not used up in the using. It is probable that the enzymes are not directly produced by the cells, but are developed from *proenzymes* or *zymogens* which the cells elaborate. The presence of zymogens in microörganisms has been very little investigated. It is probable that most, if not all, of the changes brought about by microörganisms, including the building up of protoplasm, synthetic action, and analytic action of all kinds, are directly due to the enzymes present in these cells or secreted by them.

A satisfactory definition of the term enzyme includes necessarily a discussion of enzyme characteristics.

Characteristics of Enzymes. — The *chemical composition* of none of the enzymes of microörganisms has been determined satisfactorily. Some authors even have claimed that enzymes are not to be regarded as chemical compounds at all, but simply as forms of energy. This hypothesis seems wholly improbable, however. It is certain that the enzymes are colloidal in nature, and in other respects most of the enzymes which have been carefully studied appear to be protein-like substances. The difficulty in analysis arises from the fact that a small amount of an enzyme may bring about a considerable amount of change. A quantity of enzyme too small to be weighed or to be detected in any other way can produce changes the products of which are easily observed. The enzymes are generally soluble in water and dilute salt solution. An excep-

tion is to be found in some of the fat ferments. They are partially precipitated from solution by alcohol and by concentrated solutions of ammonium sulphate, in this resembling the ordinary proteins. They are also readily adsorbed by colloids and solid particles. For example, filtration of a solution through a porcelain filter may remove a large proportion of the enzyme originally present. Precipitation of a protein in solution will carry down the enzymes at the same time. It is difficult to separate the enzymes from their impurities.

Like microörganisms, enzymes have optimum, maximum, and minimum temperatures for their activities and are destroyed by high temperatures. Usually action ceases at 0° C., the optimum for most types lies between 35° and 50° C., and they are soon destroyed at temperatures above 70° C., and almost instantaneously by boiling water. When completely dried, enzymes may withstand higher temperatures.

Enzymes are relatively unstable. They gradually lose their activity when in solution even under the most favorable conditions. Some lose their activity in a very few hours after isolation, others deteriorate but slowly. Light, particularly the violet and ultraviolet rays, is destructive.

Enzymatic actions are affected by chemicals in three principal ways. (1) The compound may act directly upon the enzyme. This may result in the destruction of the enzyme on the one hand, or a transformation to a more active form on the other. (2) The compound may affect the reaction by stimulation or inhibition of the catalysis. (3) The compound may act upon the substratum or material to be changed by the enzyme, rendering it easier or more difficult to transform. Strong acids and alkalies destroy enzymes. The optimum reaction of the medium for maximum activity varies with the enzyme, some, as pepsin, work only in acid solutions, others only in solutions that are alkaline or neutral. Enzymes are also sensitive to the action of antiseptics, though not always to the same degree as are living cells. Advantage is taken of this difference in sensitiveness to prevent the growth of microörganisms in solutions in which it is desired to study enzyme action. Great care is necessary to make certain that microbial

growth is completely inhibited or the results of the study of the enzyme will be vitiated. The antiseptics commonly used for this purpose are toluol, chloroform, and sodium fluoride. Toluol interferes but little with enzyme action. It floats on the surface of the medium. Chloroform is heavier than water and sinks to the bottom. The best antiseptic for many purposes is a 0.3 per cent solution of sodium fluoride.

Modes of Action of Enzymes. — It has been noted above that enzymes can bring about an apparently indefinite amount of change; that is, enzymes are not used up in the using and they do not form a part of the final product of their action. This does not mean, however, that enzymes never go into combination with the substance which they ferment. It is quite probable that they first combine with the compound to be transformed, bring about their characteristic change, and are later broken off and freed ready to unite with fresh molecules, and so continue this change indefinitely.

Enzymes act in one of two principal ways, by addition or removal of water, and by oxidation or reduction. These will be discussed below.

Enzymes have been defined above as organic catalysts. A catalyst is any substance which will bring about chemical change without entering into the composition of the final product. As examples of catalytic action may be cited the ignition of the hydrogen jet by means of platinum black or a platinum sponge, and the conversion of alcohol into ether by the use of sulphuric acid. Enzymes are to be considered as bringing about changes in much this same manner. However, in every instance, they are organic in origin and are relatively specific; that is, a certain enzyme will act only upon a certain compound or group of compounds and upon no others.

Reversibility of Enzyme Action. — Enzymes are useful to plants and animals not only in the digestion and breaking down of complex compounds, but also in building up the protoplasm, reserve food granules and various cell structures. Enzymes are both analytic and synthetic. It is probable that the same enzymes that under certain conditions are able to bring about digestive changes

are able under other conditions to build up complex compounds. For example, the enzyme maltase when in solution in contact with maltose converts the maltose into dextrose. Careful exam-

$$\underset{\text{Maltose}}{C_{12}H_{22}O_{11}} + H_2O + \text{maltase} = 2\,\underset{\text{Dextrose}}{C_6H_{12}O_6} + \text{maltase}$$

ination will show that this reaction never progresses to completion; that is to say, all of the maltose is never converted into dextrose. The enzyme simply brings about an equilibrium between the amount of dextrose and the amount of maltose in the solution. If a fresh solution is made containing an amount of dextrose equal to that dissolved in the other solution and maltase be added, a portion of this dextrose will be found to be converted into maltose (or isomaltose), and the equilibrium established is the same as that established by the action of the maltase upon the maltose. In other words, the enzyme maltase can build up maltose as well as hydrolyze it. This fact is of very great importance as it enables one to understand why enzymes during the life of the cell may aid that cell in building up its protoplasm and reserve food materials and under other conditions may later bring about their disintegration. Sometimes enzymes which seem primarily to function synthetically during the life of the cell may after its death bring about disintegration.

Intracellular and Extracellular Enzymes. — It has already been noted in the preceding chapter that enzymes may be divided into two classes, intracellular and extracellular. The former are present only within the cell and are never given off during its life. Even after the death of the cell it is very difficult in many cases to secure a solution of the enzyme. Extracellular enzymes, on the other hand, are excreted from the cell, and bring about their changes outside of the cell wall.

Groups of Enzymes

Enzymes may be divided into two principal classes on the basis of their method of action. These classes are subdivided on the basis of the substrate (material acted upon by the enzyme). The primary groups are the hydrolases and the oxidases.

Hydrolases and Anhydreses. — The hydrolases are typically digestive enzymes: they are frequently extracellular. The hydrolases are hydrolytic enzymes; that is, they bring about decomposition by the incorporation of water, usually resulting in a splitting into simpler molecules. A typical example is the inversion of cane sugar by invertase.

$$\underset{\text{Saccharose}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Fructose}}{C_6H_{12}O_6} + \text{Invertase}$$

The hydrolases may under other conditions catalyze syntheses; enzymes which cause syntheses by withdrawal of water are sometimes termed *anhydreses*. An anhydretic synthesis is the converse of a hydrolytic analysis. The hydrolases are subdivided into four main groups designated as the *esterases* (or *lipases*), the *carbohydrases*, the *amidases* and *proteases*, and the *coagulases*. In general these names indicate the type of material acted upon by the group of enzymes. The esterases or lipases split the esters or fats. The carbohydrases split the carbohydrates. The amidases and proteases hydrolyze or digest proteins and allied compounds. The coagulases bring about coagulation of protein or other substances in solution. These will be discussed in order.

Esterases and Lipases. — Many microörganisms produce enzymes which are capable of decomposing esters and fats. The most important of these are the true lipases which decompose fats into fatty acids and glycerol. Certain organisms living in butter, for example, produce enzymes that are capable of decomposing the stearin according to the following reaction:

$$\underset{\text{Stearin}}{(C_{17}H_{35}CO_2)_3C_3H_5} + 3\ H_2O + \text{Lipase} =$$
$$3\ \underset{\text{Stearic acid}}{C_{17}H_{35}COOH} + \underset{\text{Glycerol}}{C_3H_5(OH)_3} + \text{Lipase}$$

The initial step in the decomposition of fats is always an hydrolysis of this nature. Lipases, however, are not very common among microörganisms, being known in comparatively few species. Other esterases known to be produced by microörganisms are the *phosphatases* which hydrolyze esters of phosphoric acid, *lecithinase*

which hydrolyzes lecithin, *cholesterase* which hydrolyzes cholesterol, and *chlorophyllase* which attacks chlorophyll.

Carbohydrases. — Enzymes capable of hydrolyzing carbohydrates are produced by a very great number of microörganisms as well as by most higher plants and animals. Carbohydrates constitute a large proportion of the food of plants and animals, and the digestion and utilization of this food is usually dependent upon the presence of such enzymes. The carbohydrases may be discussed in four groups: (1) those which decompose disaccharides, (2) those which ferment trisaccharides and tetrasaccharides, (3) those attacking glucosides, (4) those hydrolyzing polysaccharides. The first and fourth of these groups are of great economic importance.

Probably in no case is a disaccharide of the general form $C_{12}H_{22}O_{11}$ utilized directly by organisms as food. These sugars are generally hydrolyzed and the monosaccharides into which they are decomposed subsequently utilized. Sometimes the enzymes are collectively termed the invertases. The enzyme *maltase* converts malt sugar or maltose into dextrose. This enzyme is produced by many bacteria and molds and by many species of yeasts. *Sucrase* hydrolyzes a molecule of cane sugar into one molecule of dextrose and one of levulose. This enzyme is produced by a few bacteria, by many molds, and by a considerable number of yeasts. The enzyme *lactase* converts lactose or milk sugar into dextrose and galactose. A comparatively small number of bacteria and molds and a very limited number of species of yeasts produce this enzyme. This sugar is therefore not as easily fermented as the two that have already been named. Other enzymes have been described from microörganisms capable of decomposing by hydrolysis various others of the disaccharides. These compounds are of relatively little importance, and their enzymes need not be discussed. The same may be said of those enzymes which decompose trisaccharides and tetrasaccharides.

Enzymes capable of decomposing glucosides (compounds which break up into glucose and other substances) are produced by many organisms, but the glucosides are not of very great economic importance, and there are few if any economic changes

brought about in them by the microörganisms. The reaction may be illustrated by the hydrolysis of amygdalin, the essential oil of bitter almonds, by the enzyme *emulsin*.

$$\underset{\text{Amygdalin}}{C_{20}H_{27}O_{11}N} + 2\ H_2O = \underset{\text{Benzaldehyde}}{C_7H_6O} + HCN + 2\ \underset{\text{Glucose}}{C_6H_{12}O_6}$$

In this case one of the products of the hydrolysis is the extremely toxic hydrocyanic acid.

The polysaccharides include those carbohydrates having the general formula $(C_6H_{10}O_5)_n$. Various members of this group are hydrolyzed by different enzymes: *starch* is decomposed by the enzyme *diastase* or *amylase*. The starch molecule is exceedingly complex and breaks down by a succession of hydrolytic cleavages into dextrins and the dextrins are for the most part decomposed into maltose.

Amylases are produced by many molds, by a few bacteria, and by none of the common species of true yeasts. These enzymes (often called diastases or simply diastase) are also very commonly found in the higher plants, particularly in the seeds, the leaves, and the portions of the plant where starch is stored as food. The conversion of starch into maltose by the amylase derived from certain molds has proved to be of considerable importance in the manufacture of industrial alcohol. Before the maltose is finally fermented, it is of course hydrolyzed, as has been indicated above, by the enzyme maltase.

The polysaccharide cellulose is hydrolized by the enzyme *cellulase*. A few bacteria, and a considerable number of species of molds, are known to produce cellulase. They are the forms which are active in the decay and decomposition of plant remains in nature. Inulin is hydrolyzed by the enzyme *inulase* into levulose. This enzyme is known to be secreted by certain molds. Inulin is a carbohydrate found in the roots and stems of a few plants. *Cytase* is an enzyme which has been found capable of hydrolyzing certain of the hemicelluloses. The hemicelluloses differ from the true cellulose in that their hydrolytic products, instead of being dextrose, are generally mannose, galactose, or one of the pentoses. They are, in other words, chiefly mannans, galactans, and pentosans. Certain bacteria and molds produce *seminase*.

Pectin is the substance which is found in the middle lamella or layer which separates cells of plants from each other. It is one of the substances which bind the cells together into a tissue. Certain molds and bacteria secrete an enzyme *pectinase* which decomposes pectin, allowing the tissue cells to be easily separated. This pectinase is essential to the process of retting of flax and hemp. The pectin binding the bundles of bast fibers together is decomposed, and these can then be removed and separated from each other. Bacteria which produce such plant diseases as black rot of cabbage and the rot of the carrot decompose the pectin of the middle lamella and cause the cells to fall apart. Enzymes are also known which hydrolyze various vegetable gums and mucilages.

Amidases and Proteases. — These include all of the enzymes which are capable of decomposing amides, proteins, and related compounds. These may be divided into the amidases, the desaminases, the ereptases, nucleases, trypsins, and pepsins.

The commonest and most important of the amidases is *urease*, which converts urea into ammonium carbonate.

$$\underset{\text{Urea}}{(NH_2)_2CO} + 2\,H_2O + \text{Urease} = (NH_4)_2CO_3 + \text{Urease}$$

This enzyme is produced by certain bacteria which actively decompose urea. It is important in agriculture, as it brings about the first of the series of changes which ultimately make the nitrogen of urea available to the higher plants as food.

The *desaminases* are enzymes which hydrolyze amino-acids. The reaction may be illustrated by the transformation of the amino-acid alanine.

$$\underset{\text{Alanine}}{CH_3 . CH(NH_2) . COOH} + H_2O \longrightarrow \underset{\text{Lactic acid}}{CH_3 . CHOH . COOH}$$

The *ereptases* or *erepsins* are enzymes which decompose proteoses and peptones to peptids, amino-acids, and ammonia. They are produced by most plants and animals and by many species of bacteria, yeasts, and molds. Probably there are many types of erepsins. The organisms which produce these enzymes are often of the putrefactive type.

The *pepsins* are enzymes which decompose proteins into proteoses and peptones. This is the initial step in the digestion and decomposition of proteins. They act best in acid solutions. The *trypsins* likewise decompose proteins but break them down into simpler compounds than do the pepsins, namely to amino-acids and ammonia. Many microörganisms, particularly bacteria and a few molds produce trypsins. They are those forms which are active in the decomposition of nitrogenous organic matter. Some of them are responsible for the spoiling of meats and other protein foods. They bring about the liquefaction of the curd in coagulated milk, the liquefaction of gelatin, the digestion of egg albumin and blood serum, and many other changes. These enzymes are also important in bringing about *autolysis* or *self-digestion*. Practically all cells contain these enzymes in small quantities and after death they decompose the proteins of the protoplasm.

Coagulases. — The two most common of the coagulases are the enzyme *thrombase*, which is the cause of the clotting of blood and the conversion of fibrinogen into fibrin, and lab or rennet, which coagulates milk by converting its caseinogen into casein (or its casein into paracasein). Another similar coagulating enzyme probably is in part responsible for the stiffening of muscles after death, the so-called rigor mortis, by conversion of the myosinogen of the muscle into myosin. A few bacteria and molds produce a rennet or rennet-like enzyme which is capable of clotting milk. In most cases these organisms also produce trypsin (or erepsin) and the clot formed by the action of the former enzyme is soon destroyed by the activity of the latter. The evidence for the enzymatic nature of rennin is not entirely conclusive. It does not show all of the characteristics of true enzymes.

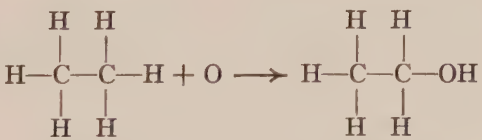
Oxidases and Reductases. — These enzymes are those which are of special importance to the cell in that they are basic in respiration and in many fermentative changes. The hydrolytic changes produced by the hydrolases discussed above release or bind relatively small amounts of energy; they are primarily digestive in function. The oxidases, on the other hand, frequently release by their action considerable quantities of energy. The hydrolytic enzymes are frequently extracellular, the oxidative enzymes are

usually intracellular, the cell making use of the energy released by their activity in synthesis, heat production, motion, light production, etc.

It should be emphasized that whenever an oxidation of a substance occurs there must be a simultaneous reduction. An oxidase is therefore also a reductase, the designation used being in general determined by whether the primary interest is in the oxidative or the reductive change which occurs. For example, microörganisms may simultaneously reduce nitrate to nitrite and oxidize a carbon compound. One might state that the reduction of the nitrate is due to the activity of a reductase, the oxidation of the carbon compound to the activity of an oxidase. Both are manifestations of the same transformation.

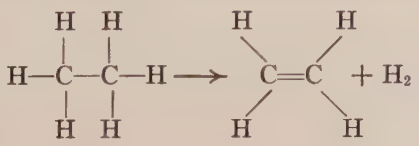
In general the nature and characteristics of the oxidases (including reductases) are less well understood than those of the hydrolases. In many cases they are freed from cells with great difficulty, or not at all. With some it is contended that the union with the protoplasm is so complete that they cannot be separated.

For the most part the active oxidases of microörganisms oxidize organic compounds, more rarely inorganic. It should be clearly held in mind that oxidation of an organic compound may be brought about in at least two distinct ways, either by the addition of oxygen to the compound or by the removal of hydrogen. For example, ethane may be oxidized to ethyl alcohol by the addition of oxygen



Ethane Ethyl alcohol

It may also be oxidized to ethylene by removal of hydrogen,



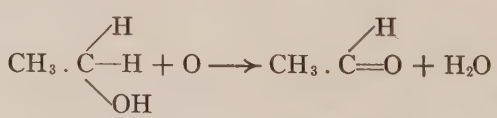
It is evident, therefore, that any enzyme which catalyzes the addition of oxygen or the removal of hydrogen is to be regarded as an oxidase, and any which removes oxygen or adds hydrogen is a reductase. It is apparent further that if hydrogen is added to one compound it must be removed from another. Any compound which under the influence of a catalyst gives up hydrogen to another is termed a *hydrogen donator*, and is, of course oxidized. Any compound which takes up hydrogen, and is thereby reduced, is termed a hydrogen *acceptor*. In some cases apparently the compound is first hydrated, that is, takes up water, and then hydrogen is removed, resulting in oxidation.

The classification of the oxidases lacks somewhat of the definiteness of that of the hydrolases. They may for convenience be separated into three groups depending on the nature of the hydrogen acceptor which is utilized.

Oxidases catalyzing Oxidation with Oxygen as the Hydrogen Acceptor. — Certain of the oxidases usually bring about their characteristic changes only in the presence of oxygen.

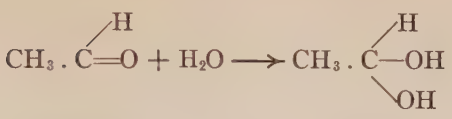
Some of these, as the *phenol oxidases*, are significant because of the color changes they produce. For example, certain bacteria when growing aërobically in a medium containing tyrosin produce a brown to black pigment, coloring the medium about the colony.

Others produce oxidative changes in alcohols and aldehydes. For example, the bacteria which produce vinegar (acetic acid) from ethyl alcohol possess the power (enzyme?) to oxidize ethyl alcohol to acetaldehyde, and this in turn to acetic acid. Free oxygen usually acts as the hydrogen acceptor. This sequence of changes may be illustrated as follows: —

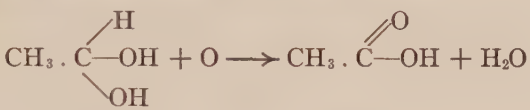


Ethyl alcohol Acetaldehyde

Aldehydes in contact with water may exist also in a hydrated form; the acetaldehyde may then take up water to become acetaldehyde hydrate: —



Oxygen may again serve as a hydrogen acceptor.



Oxidative enzymes of this general respiratory type are usually demonstrable in the activities of aërobic microörganisms.

Oxidases which catalyze Oxidation with Substances other than Oxygen acting as Hydrogen Acceptors. — It was noted previously that certain organisms which are usually regarded as aërobic will also grow under anaërobic conditions provided some readily reducible substance such as nitrate is present. Under these conditions the nitrate acts as the hydrogen acceptor, and water splits off, with a reduction of the nitrogen. Practically the result is the same as though oxygen acted directly as the hydrogen acceptor. Enzymes such as those responsible for reduction of nitrates and sulphates have not been isolated and have been but little studied.

In many cases microörganisms, particularly the aërobic types, are found to produce the enzyme *catalase*. This enzyme decomposes hydrogen peroxide into water and oxygen.

$$2\,H_2O_2 \longrightarrow 2\,H_2O + O_2$$

It has been found that hydrogen peroxide is formed by certain cells; it is also poisonous to them. Apparently the presence of the catalase removes the toxic material as rapidly as it is formed. The catalase is usually regarded as a reductase, its primary activity is that of removal of oxygen.

Many cells also produce *peroxidases*. These catalyze the simultaneous reduction of hydrogen peroxide and the oxidation of another compound.

Oxidases which catalyze Intramolecular Oxidation and Reduction. — In all the cases just enumerated there has been a reduction of one compound and oxidation of another. In other cases there may occur intramolecular oxidations and reductions. Enzymes which bring about such changes are sometimes grouped together as *zymases*. The one which has been most studied is the zymase of yeast. This is probably a mixture of several enzymes, which by their combined or consecutive actions transform monosaccharides (particularly dextrose and fructose), into ethyl alcohol and carbon dioxide. Undoubtedly there are numerous intermediate steps in the process, but attention may be focussed upon the initial and final products.

$$\underset{\text{Dextrose}}{CH_2OH . CHOH . CHOH . CHOH . CHOH . CHO} \longrightarrow 2\,C(=O)=O + 2\,\underset{\text{Ethyl alcohol}}{CH_3 . CH_2OH}$$

Carbon dioxide

An examination of the formula for dextrose shows it to contain one primary alcohol group (CH_2OH), four secondary alcohol groups (. CHOH .) and one aldehyde group (. CHO). In the products there are two molecules of carbon dioxide; the carbon in these molecules is completely oxidized, more highly oxidized than any of the carbons in the dextrose from which it is derived. On the other hand each methyl carbon group (CH_3.) in each alcohol molecule is more highly reduced than any in the dextrose molecule, the other group is a secondary alcohol group (. CH_2OH). It is apparent that there has occurred intramolecular oxidation and reduction, some carbons are oxidized, others reduced. This yeast enzyme is, like the other zymases, intracellular.

A somewhat similar enzyme is probably present in the lactic acid bacteria, the transformation in this case may be represented as

$$\underset{\text{Dextrose}}{CH_2OH . (CHOH)_4 . CHO} \longrightarrow 2\,\underset{\text{Lactic acid}}{CH_3 . CHOH . COOH}$$

Here again there is simultaneous intramolecular oxidation and reduction.

CHAPTER XXIII

MICROÖRGANISMS AND FOOD PRESERVATION

It is necessary that food be protected from the destructive action of microörganisms for considerable periods of time in order that the abundance of the summer season shall be used to supply the wants of the winter. Nature herself preserves many foods by drying and by protective coverings of various kinds. Man has added to the methods of preservation until it is now possible to keep practically any kind of food material from the period of its greatest abundance to the period of its greatest scarcity. In some cases the need for preservation is limited to a few hours or days, in other cases foods must be stored for months or for years.

Agencies Destructive to Food Materials. — Before beginning a discussion of the methods which are used in the preservation of food against destruction by microörganisms, it will be necessary to get clearly in mind those agencies which tend to bring about changes in food materials. These agencies are grouped under two general headings: first, the *intrinsic*, including those which are normally present in the food itself and have not been derived from outside sources; and second, those which may be termed *extrinsic*, that is those which enter the food at some time during the process of preparation or ripening.

To the *intrinsic agencies* belong the so-called *autolytic enzymes*. In certain fatty foods, for example, there may be normally or naturally present hydrolyzing enzymes which will bring about rancidity. In fruits and certain vegetables the enzymes which normally bring about ripening may eventually produce a condition of overripeness. Certain of the oxidizing enzymes, such as those present in fruits, may produce discoloration.

The *extrinsic agencies* capable of bringing about food deterioration are the *bacteria*, the *yeasts* and the *molds*, together with the *enzymes which they produce*. Which is most important will depend upon the character of the food and the conditions under which it is preserved. In general the *bacteria* thrive best in nitrogenous foods containing a considerable proportion of water and not too much acid; they grow best in a medium which is nearly neutral. The *yeasts* for the most part grow best in a medium rich in carbohydrates, particularly in the fermentable sugars, and preferably in the presence of some acid. Like the bacteria, they prefer a considerable amount of moisture. The *molds* will grow in media somewhat drier and of almost any type. Certain molds will grow on foods that are highly acid as well as upon those which are neutral or slightly alkaline.

Methods of Food Preservation. — Food is most commonly preserved by one of four methods: by the use of high temperatures or *heat*, by the use of *low temperatures* or cold, by the use of *preservative substances* of various kinds, and by *drying*.

Preservation of Food by Heat

All types of food not damaged or seriously changed by the application of heat may be preserved by this means. Among the foods so preserved are fruits, vegetables, meats, and fish. The process in all cases consists in the application of a sufficient degree of heat to destroy all organisms, or all of certain types of organisms, and thereafter in preserving the food under conditions such that organisms cannot gain admission. The latter is usually accomplished by sealing hermetically in glass or tin cans.

Two principal methods of heat application may be used, pasteurization or sterilization. In *pasteurization*, the food is raised to such a temperature that the organisms of certain types, but not necessarily all organisms, are destroyed. In some cases, this results in a prolongation of the time during which the food may be used, in other cases it permanently inhibits certain undesirable fermentations. *Sterilization* by heat implies the use of a temperature such that all organisms are destroyed, or at least such that all capable of growing in the food under the conditions under which it is kept,

are killed. The food in consequence, if tightly sealed, may be preserved indefinitely.

Pasteurization. — This process is ordinarily applied to milk and cream and to certain alcoholic beverages, particularly beer and wine. Milk and cream are pasteurized for several distinct purposes. First, they may be heated to destroy any disease-producing bacteria that may be present. Several states, for example, require that the skimmed milk returned by creameries to their patrons shall be pasteurized to kill any of the bacteria that may cause tuberculosis when the milk is fed to calves or hogs. Many physicians and health officers advocate the pasteurization of all milk intended for human consumption when its source is unknown or unsanitary. Many cities now require the pasteurization of all milk and cream that is not certified. Second, the pasteurization of milk may destroy most, if not all, of the lactic acid bacteria present and greatly retards, if it does not altogether inhibit, the souring of milk. It has been claimed that pasteurization for this purpose is indirectly injurious to the health of the user, inasmuch as certain of the spore-bearing putrefactive bacteria are not destroyed by the temperature of pasteurization. It has been argued further that the destruction of lactic acid forms removes all inhibition to the growth of putrefactive forms, so that, although the pasteurized milk does not sour quickly, it eventually undergoes changes which unfit it for human consumption. However, it has been shown quite conclusively that it very rarely happens that all the lactic acid bacteria are destroyed in commercial pasteurization, therefore pasteurized market milk will ordinarily sour normally. It is not probable that properly regulated pasteurization is injurious. Third, pasteurization of cream is carried out by creameries in an effort to destroy most of the bacteria present, after which it is inoculated with certain desirable lactic acid bacteria or "starters." These contribute to the flavor and aroma of the butter churned from this cream. Beer and sometimes wine are pasteurized to stop fermentation by destroying the yeasts present and also to kill organisms which might give rise to undesirable flavors.

The temperature of pasteurization is determined by the character

of the material to be pasteurized and by the object in view. In the pasteurization of milk, it is desirable to heat to a temperature high enough to destroy all pathogenic bacteria, but not to a temperature high enough to impart a boiled taste or other undesirable flavors to the milk. Sixty to seventy degrees C. for twenty to thirty minutes or 80° to 85° C. for a minute or less are temperatures commonly used; preferably, however, the temperature should not exceed 70° C. and the time should not be less than five minutes. When pasteurized in the home, milk should be heated in a closed vessel placed in water. It should be raised to a temperature between 60° and 65° C. for at least twenty minutes. The vessel in which the milk is pasteurized should be closed in order to prevent the formation of a scum upon the surface, for it has been found that this scum serves to protect microörganisms, and living pathogenic bacteria may escape destruction when embedded in it. In creamery practice, the continuous method of pasteurization is commonly used. In this process an apparatus is utilized which brings the milk quickly to the temperature of about 85° C., after which it is rapidly cooled. Milk for general use and especially that for infant feeding should not be heated much, if any, above 70° C.

Sterilization. — Food materials heated to such a temperature and for such a time as will destroy all of the living microörganisms present, and so sealed that microörganisms cannot enter, may be preserved indefinitely. The process most commonly used for this purpose is that in which the food material either before or after sterilization is hermetically sealed in containers. Meat, fruits, vegetables, and in general those foods not seriously damaged by heat and which cannot readily be preserved by drying, are utilized.

In any method of canning it is necessary that the food be *properly prepared*, placed in *suitable containers*, the *air exhausted* and the food *hermetically sealed* and *sterilized*. We are here concerned primarily with the factors which determine the time and the temperature necessary for sterilization. Some six of these are worthy of emphasis.

1. *Initial Infection.* — The first of the factors determining the time necessary for sterilization is the initial infection, its extent

and kind. It will be recalled that bacteria are not killed off instantly by heat, but that the temperature determines the *rate of death*. In general, the *larger the initial number of microörganisms to be destroyed the longer will be the time necessary to kill all*. It is also apparent that the kinds of organisms present may have some influence on time needed. The spore-producing bacteria are more difficult to destroy than the non-sporulating types. It is evident therefore that washing or other means used to diminish the initial infection with bacteria will influence the ease of sterilization.

Blanching is a process which affects the numbers of bacteria present and is commonly used preliminary to canning. The food products, usually vegetables or fruits, are dipped first into hot water, then into cold water. This process shrinks the tissues somewhat, makes them relatively firmer, and tends to set the color, and at the same time removes many of the bacteria which were present, thus reducing the initial infection and possibly thereby somewhat decreasing the time necessary for sterilization. It has been claimed by some writers that blanching also renders the bacteria which remain more readily destroyed when first heated, then cooled (or chilled) and reheated. Several tests, however, have failed to show any marked effect of this "cold shock"; blanching apparently does not make bacteria more susceptible to higher temperatures.

2. *Size and Shape of Container.* — The second factor in determining time of sterilization is the size of the food container or can. It is evident that the center of any can is that portion which last reaches the desired temperature, therefore, the larger the container the longer will it take this central portion to assume the desired temperature. The shape of the container will also determine in part the distance which heat will have to travel to the center.

3. *Ease of Heat Conduction and Convection.* — The ease with which heat may penetrate to the center of the canned material is important. Where the particles of food are solid and are surrounded by water or thin syrup, convection currents are at once set up in the water when heat is applied to the can and the interior heats relatively quickly by a process analogous to that used in a hot-water system in the heating of a home. If the material lying

between the food particles is viscous or gelatinous, or if there is not much (if any) free water, convection does not occur, and heat passes to the center much more slowly and only by *conduction*. Cans of corn heat more slowly at the center than do cans of cherries; substances like pumpkin and spinach most slowly of all. When there are no convection currents, the rate of heat penetration is practically the same as the rate of heat conduction through water.

4. *Actual Acidity.* — The hydrogen ion concentration, that is, the actual acidity of the food material, is also important. It is common experience that foods such as peaches or apples are much more easily sterilized by heat than are certain vegetables such as peas, beans, and corn. The presence of acid greatly increases the death rate of microörganisms at high temperatures. In some cases acids such as vinegar or lemon juice are added, these decrease materially the time and the temperature needed for sterilization.

5. *Agitation.* — In commercial canneries devices (agitators) are occasionally employed to keep the canned foods continually in motion during sterilization. This conduces to constant mixing of the food material and increases markedly the rate at which the heating will occur. Agitation may be regarded as greatly increasing convection, making it significant even in viscous or gelatinous foods.

6. *Temperature.* — The time required for sterilization is also influenced by the temperature to which the food is subjected. The temperature is determined by the pressure and in some cases by the constitution of the food.

Three methods of canning are in common use in which the temperature used is that which may be secured at atmospheric pressure. In the *open kettle* method the material to be preserved is heated in an open vessel, then poured into the hot, sterile containers (as Mason fruit jars) and sealed. The highest temperature reached is the boiling point of the material to be preserved. When this contains considerable quantities of sugar or other solutes the boiling point may be several degrees above the boiling point of water. This method is most effective only with acid fruits and vegetables and with pickled vegetables and fruits.

In the *cold pack* process the foods are placed in jars and heated (after packing) in boiling water or in streaming steam. The temperature of the contents of the can will consequently never rise higher than 100° C. The character of the material canned and the other factors here listed determine the length of time which must be used in order to secure sterility. With acid fruits a short time only is necessary, with vegetables such as corn and beans several hours are required. This method has been widely advocated and used in the northern part of the United States. When properly carried out the results have been quite satisfactory in some localities, in others unfavorable results have been reported.

Intermittent sterilization has been advised generally in the southern United States. The cans containing the material to be preserved are placed in water, the water brought to the boiling point and kept at this temperature for a period of an hour or more. They are then allowed to cool, and the process repeated on three or more successive days. This is the principle of intermittent sterilization such as is used in the laboratory for sugar media easily destroyed by heat. The spores present begin to germinate in the first twenty-four hours. The second heating will kill all the vegetative forms which have developed. It has been contended that repeating the heating in this manner several times will quite certainly destroy all the bacteria which may be present.

There are many who deprecate the use of both cold-pack and intermittent sterilization in the home canning of food. The most serious danger is possible failure to destroy the spores of the organism producing botulism, a type of food poisoning.

The use of the *pressure cooker* in the home, and the large processing machines in the commercial canneries have made possible the use of steam under pressure for heating and the consequent securing of much higher temperatures than the boiling point of water. Experience has developed for each type of canned food the time and pressure necessary for adequate sterilization.

Unquestionably sterilization in the pressure cooker, when properly carried out, is safer and will lead to better results in some cases than other methods, particularly in the canning of non-acid vegetables and meats.

Not all canned foods, even though they may keep perfectly well, are completely sterilized. It has been determined as the result of many studies that the microörganisms which may bring about deterioration in canned foods are: *first*, those which may enter the food as the result of defective sealing; *second*, those aërobic spore-producing bacteria which normally are unable to grow in canned foods because of the complete exclusion of air but which may find conditions suitable for growth if air is admitted due to defective sealing; *third*, anaërobic spore-producing bacteria capable of growing at normal temperatures, which occasionally escape sterilization and produce deleterious changes, frequently accompanied by evolution of gas and the development of malodorous and bad-tasting compounds. Some of these microörganisms may produce poisons or toxins (such as *Clostridium botulinum*); *fourth*, certain of the most resistant of the spores belong to the thermophiles. These spores will not germinate unless the canned food is held at a high temperature for some time. When canned foods are not adequately cooled in a commercial cannery, and are stacked away in a warehouse, they may retain their heat for days or even weeks and conditions be particularly good for the development of the thermophiles. The same may also happen when canned goods are stored in hot climates.

Microörganisms in Foods preserved by Heat. — Microörganisms may gain entrance to foods by improper sealing or they may persist through a process of attempted sterilization.

Certain types of food materials, particularly the fruits, are most apt to be attacked by molds, such as Penicillium and Aspergillus. These molds do not develop unless there is free oxygen present. They fail to develop in hermetically sealed jars. They bring about changes which render the material undesirable as food, although there is no evidence that they produce poisonous substances in appreciable quantities. Usually the mold is confined to the surface, but the decomposition products of its growth frequently penetrate and flavor the whole mass.

Vegetables and meats are commonly destroyed by bacteria. The most abundant types are those which have withstood heating because of the resistant character of the spores formed. The

organisms belonging to the butyric acid group of bacteria are relatively abundant in the soil and are present on the surfaces of most vegetables. They bring about decomposition with the evolution of considerable amounts of gas. This gas may accumulate in quantities sufficient to bulge and even to break the tin in which it is sealed. The development of such organisms renders the food wholly unfit for use. Some bacteria have been described which bring about decomposition in vegetables and meats without the evolution of gas. They give evidence of their presence by the development of peculiar odors and flavors. In many cases these gain entrance to the food after it has been sealed, and are due to defective sealing. Certain poisonous products of decomposition of canned meats have been described. These will be discussed under the heading of ptomaines.

Preservation of Food by Cold

Practically all foods can be preserved for a time by the use of low temperatures; freezing enables them to be kept indefinitely. The use of cold storage has enabled us in modern times greatly to extend the season for certain foods and to carry over surplus from times of plenty to seasons of scarcity; it has also made possible the shipping of produce and meats for long distances.

Temperatures from 0° C. to a few degrees above are commonly used for the cold storage of fruits, vegetables, and eggs, and for temporary preservation of meats. The normal activity of the living cells of the fruit or vegetable is greatly reduced by cold. The enzymes present act very slowly, and microörganisms can develop little or not at all. Eggs deteriorate gradually while stored, in part due to microörganisms contained, but also probably in large measure to the autolytic enzymes present. Meats may be held for a time at this temperature without deterioration, in fact, for a time with marked improvement in tenderness and flavor. The autolytic enzymes of the meat act but slowly at this temperature. Microorganisms, particularly certain of the bacteria, are not entirely inhibited from development, and in time will bring about decomposition.

Meats, including fish and poultry, are often frozen. Changes can

occur but slowly while the meat is in this condition. That no change at all takes place has been disputed. It seems probable that, even when frozen, certain very slow degenerative changes may occur, probably due to the autolytic enzymes present. It should be noted that some bacteria may develop at temperatures below the freezing point of water, but not if the medium which they occupy is solidly frozen.

The organisms that produce detrimental changes in cold storage foods are not well known. Probably the enzymes normally present in the foods are quite as important as the organisms that gain entrance. Food coming from cold storage, if frozen, is usually thawed before it is exposed for sale. The organisms present may develop very rapidly during this process of thawing and afterward, quickly bringing about undesirable changes. It is easily demonstrated that such food decomposes more rapidly than that which has never been frozen. The length of time that food products may be kept in cold storage without danger to the health of the consumer is still a question.

The development of various refrigerating devices in recent years has greatly extended the use of cold storage in the home as well as commercial cold storage. Much work has been done in an effort to solve the problems of food deterioration when thus stored.

Preservation of Foods by Means of Preservative Substances

Substances used as preservatives may act in one of two ways: physically, by greatly *increasing osmotic pressure;* or chemically, by *direct inhibiting action* upon microörganisms.

The chemicals most commonly used to preserve foods by increasing the *osmotic pressure* are *sodium chloride* and *cane sugar*. Neither of these substances is actively antiseptic in low concentrations. It will be noted later that preservation of foods by drying also results in some cases in increasing the osmotic pressure of solutes. Sodium chloride is used for dry salting of fish and sometimes of other meats. The flesh is packed in dry salt; this rapidly removes a part of the water, and a brine is formed. Frequently this process is repeated and the meat is packed a second time in salt. This

effectually removes enough water and produces sufficient concentration of brine so that no microörganisms can develop. A brine, made of 25 per cent common salt, usually containing 10–15 per cent cane sugar and frequently saltpeter, is used for curing meats, particularly hams. It should be noted, however, that an operation such as salting may inhibit some organisms but not others. In ham curing, for example, the salt brine is usually not so concentrated but that there is growth of certain bacteria which produce acid from the sugar and which reduce the nitrates to nitrites. Thick syrup and sugar are used for the preservation of fruits in jellies and preserves. The keeping qualities of condensed and evaporated milks are in large part due to the concentration of the milk sugar, often supplemented by the addition of cane sugar. Occasionally spoilage occurs as a result of the growth of organisms which are adapted to high concentrations. Some yeasts are known which will develop in concentrated sugar solutions or syrups.

Preservatives that inhibit the growth of organisms by their chemical action as antiseptics may be divided into two groups: those *which are produced in the food* as a result of fermentation of the food material, and *those which are added* directly to the food.

Lactic acid formed by the action of lactic acid bacteria upon sugars may develop in sufficient quantities in certain foods to preserve these indefinitely against further change. Milk which has become sour changes thereafter but slowly, particularly if kept from contact with air. The lactic acid formed is a sufficiently powerful antiseptic so that in some countries meat is preserved by keeping it in buttermilk. Sauerkraut is prepared by chopping cabbage, adding salt, and allowing the mixture to ferment. Lactic acid is formed in sufficient quantities (usually about 1 per cent) to prevent further decomposition. The preservation of ensilage is largely due also to the lactic and other acids which are formed during the process of curing. The same is true with the fermentation of dill pickles. In alcoholic fermentation the *alcohol* produced acts in a measure as a preservative, inhibiting the growth of putrefactive and undesirable bacteria for the most part. *Acetic acid* is frequently produced also (as in vinegar), and is an efficient means of preventing decomposition.

Among the chemicals that are added to foods for their preservation are *benzoic acid* and *salicylic acid* and their salts, *formaldehyde*, *boric acid*, and *borates*. *Sulphurous acid* is used in clarifying and bleaching wines and fruits. It is an active disinfectant. The smoking of meat owes its efficiency to the presence of *creosote* and related compounds which are effective disinfectants. These destroy organisms present upon the surface and penetrate to a distance sufficient to prevent their subsequent entrance. It is doubtless true that the consumption of certain of these compounds added to food is detrimental to health, but for many of the preservatives the exact degree to which this is true has not as yet been definitely established. It is undoubtedly true that in the majority of cases it is advisable to preserve food materials whenever possible without the addition of antiseptics.

Preservation of Food by Drying

For every type of food material there exists a minimum of water content that will suffice for the growth of microörganisms or for the activity of enzymes. When water is present in less quantity than this, the foodstuff may be preserved indefinitely. The exact minimum that will permit growth depends upon four factors: (1) the amount of soluble materials; (2) the kinds of soluble materials (solutes); (3) the distribution of the moisture; and (4) the nature of the organism or enzyme present.

The osmotic pressure is determined by the concentration of the solutes, that is, it varies directly with the number of molecules and ions in solution in a given volume. Most microörganisms can adapt themselves to a considerable range in concentration of the solutes of the medium, but there is a limit beyond which they cannot regain their turgor. This marks the maximum concentration of solutes in which they can develop. Fruits are usually readily preserved by drying on account of their high sugar content. Raisins, for example, will keep when relatively moist, while meat or flour containing the same amount of moisture would quickly spoil.

The chemical nature of substances in solution also influences the efficacy of drying. The concentration of acid in a food by drying

may be such as to produce a reaction that will totally inhibit growth through its preservative effect.

A food may contain a relatively small amount of moisture and yet undergo changes due to the unequal distribution. Butter usually contains about 15 per cent of water, but when kept in a warm place microörganisms (principally bacteria) multiply and produce " off flavors," and even rancidity. The water is not evenly mixed through the fat, it is present in the form of globules varying in size. Conditions in these globules are right for the development of these injurious bacteria. Butter or butter fat entirely freed from water changes very slowly. Other foods, such as raisins or other dried fruits, may contain several times as much water without injury because of its uniform distribution through the entire food mass. The drying or curing of meat, particularly that containing considerable quantities of fat, is in part accomplished by the infiltration and saturation of the surface layers with fats and oils which form a relatively waterproof exterior.

Microörganisms vary greatly in their ability to grow in concentrated solutions. Molds may develop on the surface of jellies or preserves containing a considerable percentage of sugar; certain pseudo-yeasts or torulæ develop in solutions containing 20–25 per cent of common salt.

It is evident from the preceding discussion that the amount of water that must be abstracted from a given food by drying for preservation must be dependent upon the factors enumerated. In addition, a dried or partially dried food may be sealed from the air to prevent gross contamination and to prevent moisture being absorbed due to its hygroscopic nature. The exposure of foods to direct rays of the sun or to a high temperature in the process of drying destroys many of the organisms present. The same is true when certain disinfectants, such as smoke or the fumes of sulphur dioxide, are used in the cure.

Drying or desiccation of foods may be accomplished in many ways. Exposure to the direct rays of the sun or simply to the dry air is sufficient in some climates with some foods. Artificial heat and currents of heated air are used in other instances, the temperature and rapidity of drying usually being such as will not injure

the flavor. When rapid drying at low temperatures is desired, a partial vacuum may be used; this must be resorted to where the food prepared is markedly unstable. The hydraulic or other powerful press may be used to force out the excess of moisture and thoroughly compact the product. The manufacturer of cane sugar uses centrifugal action to throw off the water. Lastly the addition of salts or sugars may abstract moisture and increase the concentration of solutes.

Most food materials containing an abundance of starch are preserved by drying. Many are sufficiently dried in the natural process of ripening. Such are the grains, the flours and meals prepared from them, and certain of the nuts such as the chestnut. It sometimes happens through unfavorable seasons for ripening or on account of excessive moisture content of the air that molds develop on such materials, and render them unfit for food. Many starchy products of the baker and manufacturer, such as biscuits, wafers, crackers, macaroni, vermicelli, yeast cakes, etc., are dried for preservation. Foods containing considerable quantities of sugar, particularly when acids are present, are also preserved by drying.

In dried fruits as much as 30 per cent of water may be present. Sirups, sorghum, and molasses, and jellies, jams, and preserves contain a higher percentage of sugar. Condensed milk is frequently sweetened by the addition of cane sugar, after the elimination of a considerable proportion of the water, usually by heating in a vacuum. This is not sterilized, but the concentration of sugar added is sufficient to preserve the milk. Unsweetened condensed milk, on the other hand, is usually sealed in cans and sterilized, otherwise putrefactive organisms would develop. Milk powder is prepared by several processes, one of which consists in forcing the milk in the form of a fine spray into a heated compartment from which the air has been partially exhausted. As it falls it dries into a fine powder which keeps well and is finding extensive use.

Foods containing a high percentage of oils and fats, such as lard, olive oil, tallow, cottonseed oil, etc., are relatively free from water and under these conditions will keep almost indefinitely. Butter contains usually about 14 or 15 per cent of water, and is therefore less stable than other fat foods. Butter fat may be preserved by

melting it and removing the water. This, of course, removes at the same time the casein and milk sugar present. The deterioration of butter is dependent quite directly upon the changes produced in these substances dissolved in the water rather than on changes primarily in the fat.

Protein foods, largely flesh foods and their derivatives, are frequently dried. The abstraction of moisture in these cases must be relatively complete. Jerked meat is prepared in arid climates by exposing strips of meat to the sun and air until dry. The rapidity of desiccation and the germicidal action of the sun's rays prevent decomposition during the process of drying. Meats and fish are frequently dried after a preliminary smoking or salting. Beef extract consists of the constituents of meat soluble in boiling water which have been concentrated to the consistency of a paste. Eggs are sometimes dried by exposure to a hot dry atmosphere, then powdered.

The choice of a method for preservation of food will depend upon the ease with which the method can be applied to the particular food, whether or not undesirable changes in composition or palatability may be produced by the treatment, and the condition under which the food is to be subsequently kept or stored before use, particularly the temperature of storage, and the exclusion of air.

CHAPTER XXIV

CHANGES PRODUCED BY MICROÖRGANISMS IN INORGANIC SUBSTANCES, PARTICULARLY IN SOILS

In an earlier chapter there were discussed the cycles of several of the elements in nature. It was emphasized that such a cycle consists in each instance of alternate building up into organic compounds and disintegration into inorganic compounds, or in some cases of alternate oxidations and reductions. It was pointed out that many of these changes are due to the activity of microorganisms. It is the purpose of this chapter to discuss some of these organisms and the changes they produce in inorganic substances (elements and compounds). Some of these changes are of considerable economic significance. Many of them are changes which occur in the soil and are of great importance in agriculture from the standpoint of crop production and soil fertility. Some changes of this group are also important in the purification of sewage and trade wastes; an understanding of them is essential to an insight into such phenomena as the self-purification of streams. In some cases the changes produced may have been of considerable geological significance.

Discussion of the transformation of organic compounds will be deferred to succeeding chapters. Here it will be advisable to consider nitrogen and certain of its inorganic compounds, sulphur and some of its compounds, and more briefly certain other elements and compounds.

Transformation of Nitrogen and Its Compounds brought about by Microörganisms

Organisms capable of bringing about changes in inorganic nitrogenous compounds may be divided into three principal groups:

first, those which are capable of fixing atmospheric nitrogen, that is, of bringing it into combination with other elements to form compounds which are utilized by the organism in building up organic compounds; second, the oxidation of nitrogen compounds; and third, the reduction of nitrogen compounds.

Fixation of Free Nitrogen. Assimilation of Free Nitrogen. — In the discussion of the nitrogen cycle previously it was noted that certain groups of microörganisms have the ability to fix or assimilate atmospheric (free) nitrogen. Among these are a few bacteria, and perhaps some molds. These organisms are of very real importance from the standpoint of agriculture. The nitrogen of the air is quite unavailable as a nutrient to plants and animals in general. Most plants require that nitrogen be assimilated in the form of ammonia or of nitrates. In other words, nitrogen that is not *in* the nitrogen cycle proper cannot be used. Nitrogen content of a soil is an important factor in its fertility. In most soils an increase in the nitrogen content is advantageous to crop yield. It is apparent, therefore, that any method which will bring the nitrogen from the air into combination so that it will eventually add to the soil nitrogen is advantageous.

Several commercial methods of fixing atmospheric nitrogen have been developed for the manufacture of nitrogenous agricultural fertilizers, and their use is increasing. However, additions to soil nitrogen are for the most part due to the activity directly or indirectly of microörganisms.

Gaseous (atmospheric) nitrogen is a relatively inert gas. It combines with other elements as hydrogen or oxygen with some difficulty. In commercial fixation it has been found always necessary to use a catalyst to expedite the reaction. In one of these commercial processes (the Haber process) a mixture of nitrogen and hydrogen under the right physical conditions are catalyzed by iron with formation of ammonia. Essentially the reaction is

$$N_2 + 3\ H_2 \longrightarrow 2\ NH_3$$

Certain kinds of bacteria when growing in a medium which contains a suitable carbon food and nitrogen only in the form of the free gas are able to build up protoplasm, which contains, of course, a con-

siderable percentage of nitrogen in combination. Inasmuch as most microörganisms are able to synthesize their cell proteins from suitable carbon compounds and ammonia, it is assumed that these organisms catalyze a change which has been termed a " biological Haber process." Positive proof that the immediate product of the catalysis is ammonia is yet lacking, but it is probable.

The question at once occurs, what is the source of the hydrogen needed in this process? As noted below, certain of the nitrogen-fixing bacteria are anaërobic, and in the presence of suitable carbohydrates they produce fermentative changes which result in the evolution of hydrogen. Such bacteria live, then, in a medium containing adequate hydrogen and usually nitrogen. However, there are aërobic organisms also which fix nitrogen. All those studied require as a nutrient a carbohydrate or some similar food. In the oxidative dissimilation of this material apparently some of the hydrogen is activated, and most of it goes to atmospheric oxygen as acceptor, with the formation of water. Quite probably some nitrogen is also activated to serve as a hydrogen acceptor, and ammonia is produced, and immediately assimilated.

The microörganisms which fix atmospheric nitrogen may conveniently be placed in two groups, those which grow symbiotically with certain other plants, and those which live free in soil or water.

Symbiotic Nitrogen-fixing Organisms. — Two types of organisms are known to live in symbiotic relationship to higher plants, certain fungi and certain bacteria.

As previously noted, many higher plants have fungi which grow on the roots, in some cases internally and in others externally. These combinations of roots and fungous hyphæ are termed *mycorhizas*. Fungi are quite commonly present upon the roots of certain trees, particularly when grown in a somewhat sterile soil. The roots of pines, oaks, beeches, etc., may harbor such organisms. Many of the plants which are native to the acid soils of peat bogs, such as the heathers and certain of the orchids, grow symbiotically with a mold. The roots of the alder and the Russian olive and related trees are generally found to produce nodules or tubercles of considerable size in which a mold or bacterium develops. It has

been suggested that all of these mycorhizas are important in taking up nitrogen from the air and in converting it into a form so that it is ultimately utilized by the plant upon which the organism is growing. This may be true in some cases. Apparently there are all intergradations between true symbiosis and true parasitism. Quite certainly in many forms the symbiotic fungus has nothing directly to do with the nitrogen metabolism of the plant.

FIG. 113. — Nodules on the roots of the sweet clover, a legume (natural size).

Two genera of bacteria (*Rhizobium* and *Mycobacterium*) are known definitely to include species which grow in symbiosis with higher plants.

Members of the genus Rhizobium occur upon the roots of all leguminous plants, such as pea, bean, vetch, alfalfa, sweet clover, peanut, locust, etc. The bacteria enter the roots through the young root hairs and cause an irritation of the tissues. These respond by the development of a nodule or tubercle in which the organisms find favorable conditions for rapid growth and development. Carbonaceous material is furnished as food to the organism by the plant, and the organism in return takes up atmospheric nitrogen, a part of which ultimately becomes available to the legume as food. The capacity of leguminous plants for taking up atmospheric nitrogen and incorporating it into their bodies in this manner through the agency of the organisms on the roots is one of the most important points in agricultural economy. Leguminous crops when grown and plowed under add appreciably to the fertility of the soil because of the very considerable contribution of nitrogen which they make.

In the cells of the root nodules the bacteria frequently assume unusual shapes, sometimes they become swollen, in other cases they are branched (Fig. 114). These were observed by some of the earlier workers before they were known positively to be bacteria. Because of their resemblance to bacteria they were termed *bacteroids;* this name is still frequently used.

FIG. 114. — *Rhizobium leguminosarum* from the roots of various legumes. *a*, normal shape; *b*, involution forms such as are generally found in the older nodules.

Even the earlier workers observed differences among the organisms producing nodules on different species of leguminous plants. It was assumed, however, that they constituted different varieties of a single species which was named *Rhizobium leguminosarum.* Some authors used the name *Bacillus radicicola.* The latter designation is not now generally regarded as correct, for the genus Bacillus includes the aërobic spore-bearing rods. The bacteria of the leguminous nodules are gram-negative rods which do not produce spores. In recent years the conviction has grown that there are at least ten or more distinct species of the genus Rhizobium. It has been found, for example, that one type will cause nodule formation only on certain species of legumes, not on others. For example, the bacteria from the nodules of the alfalfa will also produce nodules on the sweet clover, but not on the soy bean. There have been discovered a number of cross inoculation groups among the leguminous plants. The organisms characteristic of these groups may be regarded as species. They are named usually after one of their host plants, as *Rhizobium meliloti* after Melilotus (sweet clover), and *Rh. pisi* after Pisum (pea).

Leguminous plants are very widely distributed in nature. Species of legumes of one kind or another are found from the tropics to the polar regions, from sea level to above the timber line on the mountains, in moist soils and dry soils, in acid soils, neutral soils and alkaline soils. The plant family, Leguminosæ, in other

words, is cosmopolitan. Yet wherever these plants are found, examination will usually reveal the nodules and the enclosed bacteria. It is evident that growth of native legumes has had much to do with the initial building up of the fertility of virgin soils.

In some cases the kind of bacterium needed for nodule formation of a particular legume may not be present in a soil. The plant may grow provided there is a plentiful supply of nitrogen already in the

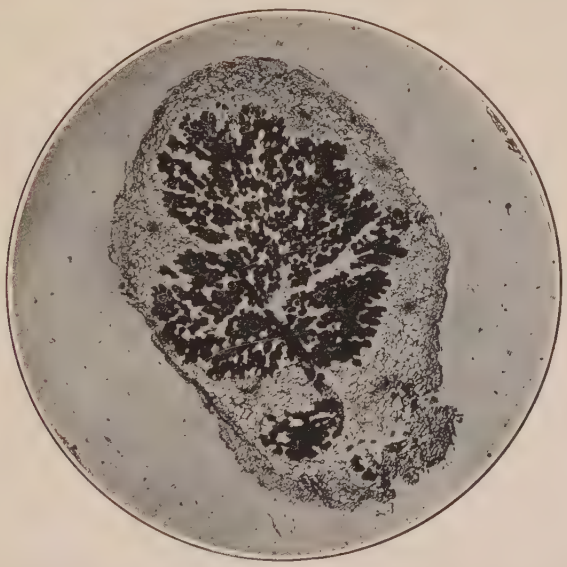


FIG. 115. — Section of leguminous nodule (from the garden bean, *Phaseolus vulgaris*) under low power objective. Note the darkly stained cells in the interior of the nodule filled with bacteria.

soil, otherwise as soon as the plantlet has exhausted the nitrogenous reserve food of the seed it will turn yellow and eventually die. It has been found profitable to inoculate seed or soil with the appropriate organisms when they are needed. This is frequently done for such agricultural crops as alfalfa and red clover. The field may be inoculated by spreading soil containing the bacteria, or, more commonly, by inoculating the seed by means of pure cultures grown in the laboratory. These may be obtained from commercial houses.

Many of the species of plants belonging to the tropical genus Rubiaceæ are known to harbor symbiotic bacteria in nodules in their leaves. The organism present is an acid-fast, non-motile-non-spore-bearing rod. It has been named *Mycobacterium rubiacearum*. It is claimed that this organism serves much the same function as do the legume bacteria in fixing nitrogen. The evidence is not as complete, however.

Non-symbiotic Nitrogen-fixing Bacteria. — The free-living nitrogen-fixing bacteria are of two types, anaërobic and aërobic.

The anaërobic nitrogen-fixing bacteria belong to the genus Clostridium. The species most studied is *Clostridium pasteurianum* (Fig. 116). It is closely related to the group of butyric acid bacteria. It has been found to be one of the more efficient of the nitrogen-fixers when studied in the laboratory, but it is not entirely clear that conditions are often right in the soil for its activity.

FIG. 116. — *Clostridium pasteurianum*, cells without and with spores. (Adapted from Winogradsky.)

The aërobic bacteria of this group are for the most part included in the genus Azotobacter. Several species have been described (as *Azotobacter chroococcum*, *A. agilis*, and *A. beijerinckii*). They are rather plump, rod-shaped bacteria, non-spore forming, often motile. They grow readily on laboratory media containing carbohydrates or similar compounds (as mannitol) and utilize atmospheric nitrogen. They are quite abundant in many fertile soils, and are probably in a measure responsible for the accumulation of the nitrogen in the soil.

It should be understood that most, if not all, of the nitrogen fixed by these free-living soil forms is not at once freed and made available to higher plants. For the most part, at least, it is built into protoplasm, and only becomes available upon the death and disintegration of the bacterial cells.

Oxidation of Ammonia and Nitrites. — In the discussion of the nitrogen cycle it was noted that the ultimate nitrogenous decomposition product of proteins was in all cases ammonia. This is afterwards converted into nitrites and then into nitrates by certain species of bacteria. This fact is of considerable importance from

two points of view, first, that of agricultural practice, and second, in the recognition of pollution of water and water supplies.

Most green plants require nitrates as a source of nitrogen in the soil. Ammonia produced by the decomposition of manures and organic matter is not directly available to many of them. This ammonia is changed in the soil by two or three species of bacteria, the Nitrosococcus and the Nitrosomonas, according to the following equation:

$$2\ NH_3 + 3\ O_2 \longrightarrow 2\ HNO_2 + 2\ H_2O$$

Nitrites are even less available to green plants than ammonia, and when they accumulate in any considerable quantity are distinctly poisonous. They are, however, ordinarily converted at once in the soil into nitrates by the action of species of the genus Nitrobacter. The reaction may be represented by the following equation:

$$2\ HNO_2 + O_2 \longrightarrow 2\ HNO_3$$

The nitrous and nitric acids do not usually exist uncombined in the soil, but as nitrite and nitrate. The latter may then be taken up by green plants.

The same changes which have been discussed as occurring in soil occur also in water which has become contaminated with organic matter such as sewage. The presence of ammonia in water is generally held to indicate a comparatively recent contamination with some organic matter. When the nitrogen is present in the form of nitrites, it is understood that oxidation has been partially effected or that nitrates have been partially reduced by fresh contamination. The presence of nitrogen as nitrates only in water indicates that some time in the past the water has been contaminated, but that complete oxidation has occurred. By studying the relative proportion of ammonia, nitrites, and nitrates in a sample of water the chemist is frequently much aided in forming a judgment as to the potability of such water.

These processes of nitrification are also of major importance in the purification of trade wastes and of sewages.

Reduction of Nitrogenous Compounds. — In the presence of an abundance of organic matter and in the absence of free oxygen, certain bacteria are capable of reducing nitrates to nitrites, and

nitrites to free nitrogen gas. This process is termed *denitrification*. It may occur to a certain extent in food materials which have been preserved by the use of saltpeter. It may also occur in soils which are waterlogged. It has been found, for example, that the addition of nitrates to a soil kept flooded for the growing of a crop such as rice is actually detrimental. Because of the anaërobic condition established in such a soil, the nitrates are converted into nitrites and act as poisons. It is found that crops grown under such conditions take up their nitrogen from the soil in the form of ammonia and related compounds.

The bacteria which are able anaërobically to reduce nitrates are quite common. They include species belonging to many genera. Bacteria able to reduce with production of free nitrogen belong for the most part to the genus Pseudomonas.

Transformation of Sulphur and Its Compounds

As with nitrogen, so with sulphur one finds both oxidation and reduction being brought about by the activity of microörganisms.

Oxidation of Sulphur and Its Compounds. — Hydrogen sulphide is oxidized by certain water and soil bacteria such as Beggiatoa with the production of free sulphur and sulphuric acid. Organisms of this type are very common in sulphur springs and in sewage which contains considerable quantities of hydrogen sulphide. The sulphur granules may easily be observed by an examination of the organisms under the microscope. They will be noted as refractive granules lying within the cell. This oxidation of hydrogen sulphide to free sulphur and sulphuric acid undoubtedly enables the organism to manufacture its food by means of the energy thus secured.

The production of this acid under certain conditions, as in contact with concrete, may prove destructive or injurious. This has happened, for example, in some cases in the concrete tanks used in sewage disposal. On the other hand, the oxidation of sulphur in contact with insoluble phosphates (as tri-calcium phosphate) in the soil may give rise to the formation of soluble phosphates, increasing thereby soil fertility.

Reduction of Sulphur Compounds. — In the presence of organic matter and in the absence of oxygen, certain organisms are known

to be able to reduce sulphates to sulphides. This is of importance in the disposal of sewage in localities where the water supply contains considerable quantities of sulphates. Such sewage carries organic matter in considerable quantities, and anaërobic conditions are quickly established. The hydrogen sulphide developed under those conditions may be sufficient in quantity to create a decided nuisance. Similar effects sometimes obtain when sewage is allowed to flow into sea water, because of the considerable amount of sulphate contained in the latter. The organisms capable of bringing about such changes are, so far as known, bacteria, principally spirilla and bacilli.

Transformation of Phosphorus and Its Compounds. — Microorganisms in the soil are active in bringing about certain transformations in phosphorus compounds, particularly in the phosphates. Certain of the insoluble compounds of phosphorus are converted into soluble forms which may be utilized by higher plants as nutrients. It is probable that the transformation is largely indirect, that is, the organisms do not directly attack the phosphates, and the changes are brought about by the decomposition products of organic matter in the soil.

Oxidation of Carbon. — It has been found that pure carbon in the form of charcoal may be oxidized by certain soil bacteria. Some of the changes which occur in coal when brought in contact with air are probably due to the development of microörganisms.

CHAPTER XXV

CHANGES PRODUCED BY MICROÖRGANISMS IN NON-NITROGENOUS ORGANIC SUBSTANCES. ALCOHOLIC FERMENTATION

MICROÖRGANISMS bring about many changes in non-nitrogenous organic substances. Several of these are of sufficient importance to warrant discussion in detail. Most important are the alcoholic fermentations of sugar; the production of lactic and propionic acids from carbohydrates; the oxidation of alcohol to acetic acid; the production of butyl alcohol and of butyric, citric, oxalic, and other acids from carbohydrates; the fermentation of cellulose, hemicellulose, pectins, and gums; the inversion of the higher sugars; the hydrolysis of fats; fermentations of the fatty acids and various syntheses. The present chapter will deal only with the production of ethyl alcohol by microörganisms, and with certain related fermentations.

Types of Organisms concerned in Alcoholic Fermentation. — All three groups of microörganisms, bacteria, yeasts, and molds, contain species which are capable of producing alcoholic fermentation of sugars in solution. Relatively few bacteria can bring about this change, and none of them is of any considerable economic importance in the manufacture of alcohol. Certain of the molds, particularly those forms which simulate the yeasts in their growth in sugar solutions, are capable of inducing alcoholic fermentation. It will be noted that some of the organisms which can produce alcohol under suitable conditions are of great importance in the fermentation industry because of their ability to saccharify starch. Such are certain species of the genus Aspergillus, particularly *A. oryzæ* and certain of the Mucors, particularly *M. rouxii*. These

produce diastase (amylase) in large quantities. Under anaërobic conditions they are capable of forming small quantities of alcohol.

By far the most important group of organisms to be considered in alcoholic fermentations is that of the yeasts. In Chapter VI it was noted that several systems of classification of yeasts have been adopted. Practically all of the important alcohol-forming yeasts belong to the genus Saccharomyces. The differentiation of species is based largely upon the type of fermentation produced, the morphology of the cell, spore production, and the capacity of the organism to ferment various sugars. The sugars which are most commonly employed to test the action of yeasts in alcoholic fermentation are dextrose, maltose, sucrose, and lactose. The yeasts are frequently classed with reference to their ability to ferment each of these sugars. Nearly all the forms can ferment dextrose. Most of them can ferment maltose. A smaller number, but still a considerable proportion, can ferment sucrose, while a still smaller number, or relatively few, can ferment lactose or milk sugar. It will be noted that different species are usually responsible for the fermentation of different saccharine solutions.

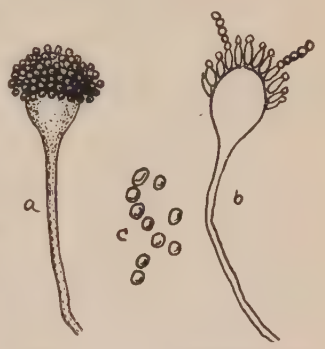


FIG. 117. — *Aspergillus oryzæ*, conidiophores and conidia. (Adapted from Wehmer.)

FIG. 118. — *Mucor rouxii*, sporangiophores, sporangia, and spores. (Adapted from Wehmer.)

Enzymes concerned in Alcoholic Fermentations. — Several enzymes will be found to be indirectly and directly concerned with the production of alcohol. Certain *diastases* are commonly responsible for the formation from starch of the sugar upon which the yeast is to act. These diastases may originate in one of several ways. They may be produced by seeds in the process of germina-

tion, or by molds or by bacteria. The former is the one most commonly used in the process of malting and brewing, while the second is sometimes used in the preparation of sugars to undergo fermentation in the production of commercial alcohol. *Invertases* are also important, inasmuch as the yeasts ferment directly only the monosaccharides. Many of the yeasts themselves produce some invertases, as *sucrase*, *maltase*, and even *lactase*, which are capable of inverting or hydrolyzing cane sugar, malt sugar, and milk sugar respectively. In some cases molds or bacteria are in part responsible for the inversion of the disaccharides. The enzyme *zymase* is the one believed to be actively concerned in the transformation of sugar into carbon dioxide and alcohol.

For many years alcoholic fermentation by yeast was the stock example cited of a change brought about by an organized ferment. It was thought that the production of alcohol and carbon dioxide was a part of the vital processes of the cell and could not be dissociated from life. In 1897 Buchner succeeded in extracting an enzyme (which he termed zymase) from the yeast cell and he proved that it could bring about alcoholic fermentation in sugar solutions. The method of preparation is to mix brewer's yeast with an equal weight of quartz sand and some infusorial earth. This is ground for some time in a mortar, a small amount of water added, and the juice pressed out by the use of an hydraulic press, and clarified by filtration. It is found upon chemical examination to contain a considerable proportion of protein material. This liquid may be proved to contain zymase by mixing with sugar solution, and after a short time carbon dioxide will be given off and alcohol formed. This yeast juice rapidly loses its power to produce changes, so that in a few days it is wholly inert. The literature treating on the subject of zymase, its preparation, and the changes which it brings about has become very extensive, and the phenomenon has been proved to be very much more complex than was originally supposed. It is known that phosphates are essential to this fermentation, and it is believed that the zymase itself is not a simple compound, but a mixture of two compounds neither of which can act alone, but if mixed can produce this typical fermentation.

Zymase, then, may be regarded as an enzyme mixture. The

first to act upon the simple sugars is that which causes the union of phosphoric acid and hexose, to form a hexose-phosphate. This compound is then broken down, and the sugar split to a carbon compound with three carbons in the chain. This is probably then transformed in normal alcoholic fermentation to pyruvic acid ($CH_3 . CO . COOH$). Yeast (as a part of its zymase complex) contains the enzyme *carboxylase*, an oxidase. This enzyme attacks specifically keto-acids, particularly those in which the keto group is in the alpha-position. It transforms pyruvic acid to acetaldehyde and CO_2.

$$\underset{\text{Pyruvic acid}}{CH_3 . CO . COOH} \longrightarrow \underset{\text{Acetaldehyde}}{CH_3 . CHO} + CO_2$$

This accounts for the production of the carbon dioxide of the alcoholic fermentation. The acetaldehyde is reduced to ethyl alcohol by acting as a hydrogen acceptor to one of the intermediate products.

$$\underset{\text{Acetaldehyde}}{CH_3 . CHO} + 2\,H \longrightarrow \underset{\text{Ethyl alcohol}}{CH_3 . CH_2OH}$$

The whole reaction may be summarized as

$$C_6H_{12}O_6 \longrightarrow 2\,CH_3 . CH_2OH + 2\,CO_2$$

As will be noted later, it is possible to modify the course of the fermentation in various ways, and secure considerable yields of other substances such as glycerol.

Alcoholic Fermentations of Economic Importance

The alcoholic fermentations of economic importance may be classified under several heads and discussed separately. These are the alcoholic fermentation of fruit juices, the alcoholic fermentation in brewing, the alcoholic fermentation of milk, the production of distilled liquors and alcohol for commercial purposes, fermentation of miscellaneous alcoholic beverages, such as pulque, ginger beer, etc., and the panary fermentations or production of carbon dioxide and alcohol in bread making.

Alcoholic Fermentation of Fruit Juices. — Practically all fruit juices containing sugars will undergo spontaneous alcoholic fermen-

tation if pressed out and allowed to stand for a time. The most important of the beverages produced in this way are wine, cider, and perry from the juice of the grape, the apple, and the pear respectively. The juices of many other fruits are occasionally used, such as those of raspberries, elderberries, blackberries, etc.

The juice of the grape (must) contains considerable amounts of sugar, principally dextrose and fructose, sometimes as much as 25 per cent. In addition there is sufficient tartaric and other acids to inhibit the growth of most species of bacteria. The juices of the apple and of the pear usually contain between 15 and 20 per cent of sugar and sufficient acid (largely malic) and tannin to inhibit the growth of most undesirable organisms.

The microörganisms primarily responsible for the fermentation of these juices are yeasts, in most cases forms which occur upon the surface of the ripe fruits. These are present in sufficient quantities so that usually the addition of yeast is unnecessary for starting the fermentation. The yeasts may be demonstrated to be present upon the surface of the ripe fruits by various means. It is not certainly known how they reach this point, as they do not occur usually upon the surface of the unripe or green fruits. It is probable that insects are largely responsible for their distribution. Yeasts generally occur in small numbers in most soils, and such yeasts gaining entrance through a puncture in the surface of the grape multiply and form a considerable colony. Flies, bees, wasps, and other insects feeding upon this sugary material would carry away considerable numbers of the cells, and in their flight from fruit to fruit serve to distribute them. It is true that false yeasts and molds are even more abundant than true yeasts upon the surface of these fruits, but under the conditions under which the fermentation usually occurs, the true yeasts soon gain the ascendancy and prevent the development of other forms. Occasionally the false yeasts are present to the exclusion of others, and abnormal fermentations will then take place. The wine yeasts are generally included in the group formerly known as the species *Saccharomyces ellipsoideus*. Careful investigations in the classification of various types of wine yeasts are still needed, so that it is almost impossible to differentiate between species accurately.

The microörganisms responsible for the fermentation of cider have been studied even less than those of wine.

The fermentation of fruit juices generally requires that some oxygen be admitted, at least in the initial stages of the fermentation, to allow of sufficiently rapid multiplication of the yeast cells. After the fermentation has started, air is largely excluded, however. After fermentation is complete, it is frequently necessary to clarify by the addition of gelatin or albumin or at least by allowing the material to stand until the yeast cells and other suspended particles have settled out. These fermented fruit juices usually contain sufficient quantities of alcohol to inhibit the growth of most organisms, provided the oxygen of the air is excluded.

Beverages produced from fruit juices, such as wines and cider, may be attacked by certain undesirable organisms and materially changed. The acetic acid bacteria will develop whenever there is sufficient exposure to the oxygen of the air. These grow usually upon the surface of the liquid, oxidizing the alcohol to acetic acid. Other organisms, known collectively as mycodermata or false yeasts, and yeast-like fungi may grow upon the surface of the solution in the presence of oxygen and oxidize the sugars, acids, and alcohol present to water and carbon dioxide, rendering the beverages insipid. When alcohol is not developed in sufficient quantities, certain kinds of bacteria may produce undesirable fermentation in the must or in the wine. Certain of the lactic acid bacteria may produce souring by conversion of sugar into lactic acid. Butyric acid bacteria, slime-producing bacteria, and molds may also destroy the product. In addition to the development of alcohol and carbon dioxide, there is more or less transformation of the protein compounds that may be in solution by proteolytic enzymes derived either from the fruit or from the development of yeasts. Changes in the color may be due to oxidases present. Undoubtedly the flavors characteristic of the wines of certain districts are largely due to the formation of certain by-products of fermentation by enzymatic activity.

Alcoholic Fermentation in Brewing. — The preparation of malt liquor or beer, with its varieties of porter, lager, and ale, may be

divided into several stages, the processes of malting, mashing, fermenting, and aging.

Malt is usually prepared from barley, but other grains are sometimes used. The grain is soaked in water for one to three days at a temperature low enough to discourage mold growth. It is then placed in vats or cylinders to germinate. It is occasionally stirred or mixed to aërate and prevent heating. The germination is continued until the sprout reaches half the length of the grain and is then stopped by drying with currents of warm air, the temperature being gradually raised as the drying progresses. When completely dry, the sprouts are removed by friction, and the product is termed malt. The process of malting is essentially one for the purpose of developing certain digestive enzymes, principally *diastase* (*amylase*), capable of converting starch into dextrins and sugar, certain *proteolytic enzymes*, and probably *cytase*. These enzymes are developed in the grain during the process of germination, and function normally to convert the insoluble materials of the endosperm of the seed into soluble substances that can be utilized by the embryo plant as food. The process of germination is arrested in malting at the point where the maximum amount of enzyme has been produced and there is a minimum amount of change in other constituents; also before any considerable amount of the stored food has been utilized by the young plant.

In the process of mashing, the crushed or ground malt is mixed with warm water and extracted for several hours. The amylase (diastase) rapidly converts the starch into dextrins and maltose, and the proteins of the grain are in part dissolved and in part digested by the proteolytic enzymes. This extract of malt is called *wort* or *beerwort*. It is removed from the solid materials and boiled to sterilize it, to aid in clarification, and in some cases to influence ultimate flavor. Hops are frequently added for the twofold purpose of contributing a characteristic flavor and aroma and to add certain antiseptic substances that aid in preservation and in prevention of abnormal fermentations.

The fermentation of the wort and its conversion into beer are initiated by the addition of yeasts. In modern practice this is usually accomplished by adding a relatively pure culture of some

one of the species or varieties generally included under the term *Saccharomyces cerevisiæ*. Where pure culture methods are not employed, other yeasts may be active, such as *S. ellipsoideus* and the harmful *S. pasteurianus*. The fermentation is carried out in vats. It proceeds with considerable rapidity for several days, then gradually subsides. The sugar and dextrins originally present in the wort are rapidly converted into carbon dioxide and alcohol. The beer is then placed in large casks, where it slowly continues to ferment, the yeast settles out, and the aging is complete in from a few weeks to several months.

Bacteria of the lactic acid type if not controlled may cause souring of the wort. Occasionally butyric acid bacteria may develop. Acetic acid bacteria cause deterioration of the beer when opened. Certain torulæ and wild yeasts may gain entrance and produce disagreeable flavors, and slime-producing bacteria may form gummy dextrans and injure both consistency and flavor. Pasteurization is usually resorted to for the preservation of bottled beers.

Non-alcoholic malt beverages (" near beer ") are prepared in the same general manner as beer, the alcoholic content being reduced to less than one-half per cent by distillation.

Alcoholic Fermentation of Milk. — Milk usually undergoes lactic acid fermentation, as there are few organisms capable of producing alcohol from lactose, probably because of the usual absence of the enzyme lactase. Milk (more frequently cream) may undergo alcoholic fermentation following lactic fermentation. It is not usual for the lactic acid bacteria to ferment all of the lactose present in the milk or cream. The more slowly developing yeasts are not inhibited by the concentration of the lactic acid which stops the growth of the bacteria. Yeasts may then produce an alcoholic fermentation. Cans of cream, for example, shipped without refrigeration during hot weather may even blow off the sealed lid through the pressure of the accumulated gas. The development of the yeasts imparts an undesirable flavor and aroma, and may prove a serious difficulty in the manufacture of a high-grade butter. Alcoholic beverages from milk are either produced through the activity of a lactase-forming yeast, through the inversion of the lactose by some other organisms present, or by the addition of some

sugar such as sucrose which can be fermented by the commoner types of yeasts. In all cases more or less lactic acid fermentation occurs simultaneously with the alcoholic, the inoculating material used and the temperature being the factors that determine the relative proportion of acid and alcohol.

A modified *koumiss* is sometimes prepared by the addition of cane sugar and compressed yeast to milk; this is kept at blood heat until vigorous fermentation begins, then placed in tightly corked bottles and chilled. The lactose is utilized little or not at all in the alcoholic fermentation, as the common bread yeasts produce no lactase. This fermentation results in the production of a beverage having a low content of alcohol (about 1 per cent) and acid (about .75 per cent), and one that is markedly effervescent. It is sometimes prescribed in the dietary of invalids and convalescents.

Koumiss proper is a beverage prepared originally by certain natives of southern Russia from mares' milk. Inoculation is made from a previous lot of koumiss. The fermentation in this case is due to the combined action of yeasts and bacteria, the former of the lactose-fermenting group.

Kefir is a beverage produced by the natives of the Caucasus. The milk is fermented as the result of inoculation with "kefir

FIG. 119. — Kefir grains, natural size. (Adapted from Freudenrich.)

grains" (Fig. 119). These are dried zoögloeal masses of various sizes consisting of a mixture of yeasts and bacteria. These kefir grains are fished out and preserved for future use after having initiated the fermentation. The product of the fermentation is a beverage containing about one per cent of acid and a small amount of alcohol. The yeast is called *Saccharomyces kefir*, differing from

S. cerevisiæ in its fermentative powers. *Leben* is a somewhat similar beverage prepared in Egypt. *Yogurt* or *mazun* is a much more acid beverage prepared in Bulgaria. It will be discussed under the heading of lactic acid fermentation.

It will be noted that these beverages prepared from milk will keep longer than will the unfermented milk. In tropical countries particularly, the use of such beverages has long been practiced because of the difficulty of keeping milk.

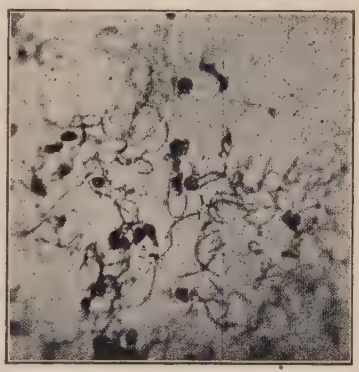


FIG. 120. — Smear from an active kefir grain that has been causing fermentation of milk. Note the slender bacilli and the yeast cells. (× 500.) (After Kuntze.)

Not only the sugars, but the proteins of the milk as well are usually somewhat changed in alcoholic fermentations, being partially digested. It has been urged that this fact makes such foods particularly useful for those that have difficulty in digestion and assimilation.

Alcoholic Fermentation for Distilled Liquors and for Commercial Alcohol. — Beverages of many types are produced by the distillation of fermented saccharine solutions. *Brandy* is prepared by the distillation of fermented fruit juices, *rum* from fermented molasses or cane sugar syrups, *whisky* by the distillation of a fermented mash prepared from malted grain.

Commercial alcohol may be prepared by distillation from fermented saccharine solutions of various kinds. The chief problem in its manufacture is to secure a satisfactory and cheap source of sugar. This is accomplished by the use of the saccharine juices of plants such as the sugar beet, the waste products of cane sugar manufacture, and chiefly by the saccharification of starch.

Three methods for the conversion of starch into sugar for alcoholic fermentation have been used. It is possible to convert the starch of grains and potatoes, etc., into sugar by heating with sulphuric or

other acids. The starch is broken down into glucose, the excess of acid neutralized, and the mixture fermented by the inoculation of yeasts. Grains may be malted as has already been described under the heading of brewing. It is not necessary to use the same care, inasmuch as the product is not to be used directly as a beverage and grains that are not suitable for brewing purposes may be used in this manner. The third process that has been somewhat utilized is that of saccharification by means of certain molds. Several species have been described that produce considerable quantities of amylase. This process of saccharification is called the *amylomyces* (literally *starch fungus*) process. Several species of Mucor, particularly *M. rouxii* and *M. oryzæ*, and one species of Aspergillus, *A. oryzæ*, have been utilized for this purpose. The *Mucor rouxii* is the characteristic mold of *Chinese* or *Java yeast* or *ragi*. This yeast is prepared by mixing bits of sugar cane, certain root stalks, and rice meal. This is ultimately allowed to ferment and the residue made up into flat, round cakes which are dried in the sun. These are utilized in the saccharification of starch in rice. Rice is boiled until it is a semifluid, pasty mass. It is mixed with the powdered Chinese yeast and allowed to stand for two days. During this time the molds inoculated by their diastatic action change the starch into sugar. The utilization of the mold in this manner suggested the possibility of saccharifying other types of starch than rice flour by its use.

In the amylomyces process the starchy material, usually corn or some other grain, is steamed for several hours under three to four atmospheres of pressure. It is then cooled and placed in a fermenting vat. A culture of Mucor or Aspergillus is introduced and thoroughly mixed with the contents of the vat. Sterile air is then allowed to bubble up through the mash. In the course of twenty-four hours, the entire mass has been permeated by the mycelial threads of the fungus, and saccharification induced. The time when conversion of the starch into sugar has been completed is determined by the use of the iodine test. The current of air is then stopped, yeast is added, and this together with the Mucor converts the sugar into alcohol and carbon dioxide. When the content of alcohol has reached its maximum, the mash is distilled.

Attempts have been made to develop satisfactory methods for saccharifying sawdust and similar waste wood products in order that they might be used in alcoholic fermentation. The methods developed are apparently feasible, but the yield of alcohol is inadequate to make it profitable.

Fermentation of Other Alcoholic Beverages. — Many other beverages than those already enumerated are produced by alcoholic fermentation of various saccharine solutions. In fact, practically every section of the globe has its own peculiar product of this type. The juice of certain palms collected by cutting off the terminal bud yields *palm wine*. The sugary juice of the agave is used in Mexico for the production of *pulque*.

FIG. 121. — *Lactobacillus vermiformis*, various types of cells with irregular capsules, from ginger beer. (Adapted from Ward.)

Ginger beer is prepared by adding ginger and sugar to water and inoculating with what is termed ginger beer plant. This is an impure culture preserved in much the same manner as are grains of kefir for the fermentation of milk. Ginger beer is characteristically somewhat viscous. It is produced as the result of the symbiotic growth of a yeast and a bacterium which form lactic acid and more or less gum. The organisms causing this fermentation are *Saccharomyces piriformis* and *Lactobacillus vermiformis*.

The beer called *saké* or *rice beer* is produced by the Japanese. This Japanese beer is of interest because saccharification of the rice used in this preparation precedes the fermentation by yeasts. It is brought about by the *Aspergillus oryzæ*, which has been mentioned above as sometimes useful in the production of commercial alcohol.

Pombe is produced in certain African countries by the fermentation of a malt prepared from millet seeds. It is particularly interesting inasmuch as the fermentation is brought about by a member of the genus Schizosaccharomyces, *S. pombe*.

Mead is produced by the fermentation of honey diluted with water, usually with the addition of certain salts.

Panary Fermentation. — The change which occurs in flour and in the other constituents of dough before baking into bread may be termed panary fermentation. It consists essentially in the production of gas, usually as a result of alcoholic fermentation brought about by yeasts. The preparation of the various yeasts or leavens will first be considered, and this followed by a discussion of the changes they bring about in bread making and of certain abnormalities in bread due to undesirable micro-organisms.

Preparation of Leaven. — The leavens used for bread making may be divided into three groups, the relatively pure yeasts secured in the form of brewer's yeasts, compressed yeasts, or yeast cakes; the leavens propagated in the bakery or the home in solutions of various kinds (the "barms"); and the spontaneous leaven that develops in mixtures of flour and water under appropriate conditions.

Brewer's yeast skimmed from the surface of the vats of fermenting wort is sometimes used in bread making. It spoils easily, must be kept constantly cold, and at the best must be used within a short time after preparation. The compressed yeast most commonly used by bakers is a somewhat more concentrated product, and may be kept in the cold for several days without deterioration. It is prepared in one of two ways, the so-called *old* or *Vienna process* and the *new* or *aërating process*. In the old process a mash is prepared from grains such as rye, barley, maize, buckwheat, in some cases with the addition of potato. The grains are crushed, malt is added and the mixture soaked in water at 44–50° C. This gives the optimum conditions for saccharification, and the diastase soon converts a large proportion of the starch into sugar. Frequently this is sterilized by heat to prevent growth of undesirable forms. It is acidified by the addition of acid mash or by inoculation and fermentation with one of the lactic acid bacteria. It is then inoculated with yeast, usually one of the types of *Saccharomyces cerevisiæ*. An active fermentation ensues and the yeast forms a heavy mat on the surface of the

fermenting liquid. This is then removed and by means of a sieve separated from any coarse particles. It is suspended in cold water (about 10° C.) and allowed to settle out; the water is drawn off and the yeast mass partially dried by pressure and forced into cakes. In the new process a wort is prepared much as in brewing by the saccharification of various grains or potatoes by malt, followed by a filtration of the sugary extract. In some cases molasses is diluted

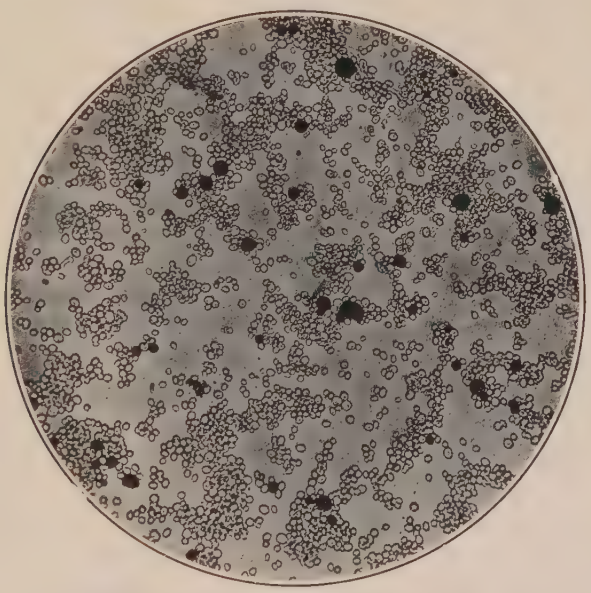


FIG. 122. — Compressed yeast under low power. Starch grains have been colored by iodine. Note that they are very few in comparison with Fig. 123.

and substituted for the wort. The acidity essential to prevent the growth of undesirable organisms is secured by inoculating with a lactic acid organism (as *Lactobacillus delbruckii*) and holding at a relatively high temperature (50° C.) for a few hours. The liquid is cooled to 30° C., inoculated with a pure culture of yeast, and air bubbled through for a period of six to eight hours. Growth of the yeast is very rapid. It may be removed by centrifugation or by cooling and allowing it to settle to the bottom. It is washed and prepared as in the old process.

It is customary to mix starch, flour, or meal with the yeast before it is pressed into cakes, though with improved methods this is not strictly necessary. When a considerable proportion of meal is used, the product may be dried; such are the yeast cakes commonly purchased. Yeast in a dried cake slowly loses its vitality; an old yeast cake, particularly one which has not been kept cool, may be wholly unable to initiate fermentation.

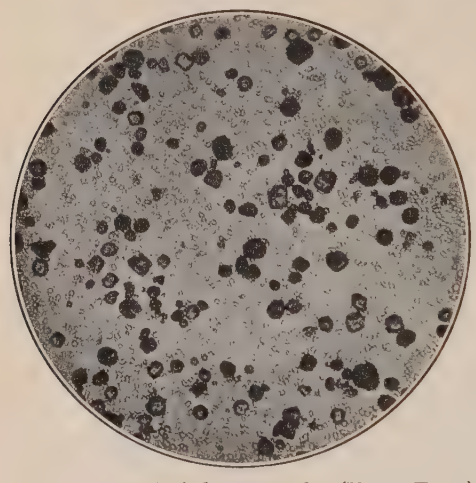


FIG. 123. — Dried yeast cake (Yeast Foam) under low power. Starch grains dark. Note the starch constitutes a large proportion of the yeast cake.

The leavens used for bread not only cause production of the essential gas, but also contribute more or less to the flavor as well. The latter is especially true if they contain a certain proportion of microörganisms other than the yeast, particularly lactic acid and gas-producing bacteria. The continued propagation of these organisms may be accomplished by holding a portion of the dough from one baking to be used in the next. Not infrequently, especially in England and Scotland, and to a less degree in the United States, these organisms have been propagated in special mixtures. Until recent years practically every locality and every successful bakery had its own particular formula for the preparation and propagation of the leaven, or barm. The usual constituents are water, potatoes, sugar, and hops. The hops are instrumental both in eliminating undesirable fermentations and in contributing

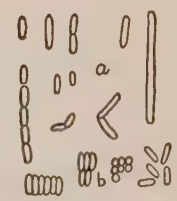


FIG. 124. — *Bacterium panis fermentati*, one of the organisms capable of producing gas in dough. (Adapted from Henneberg.)

a desirable flavor. These mixtures are ordinarily allowed to ferment spontaneously, although sometimes a little yeast is added to initiate the process. The composition of these barms unquestionably modifies materially the flavor and texture of the bread.

Flour usually contains of itself sufficient bacteria and yeasts so that when mixed with water gas will slowly develop. Undesirable organisms may be in part inhibited by the addition of salt and the control of the temperature. Such is the source of leaven in the so-called salt-rising of sour breads.

Fermentative Changes in Bread Making. — The essential in the preparation of all bread is to secure sufficient production of gas to cause the dough to rise, or become light. Generally certain characteristic flavors and aromas develop also in consequence of this fermentation. The gas is usually the result of alcoholic fermentation of sugars by the yeasts present, but it is probable that in some cases bacteria also play an active part.

Flours and meals for the preparation of bread are usually made from wheat or rye, occasionally from maize or barley. They are all high in carbohydrates, and the first two contain a considerable proportion of protein, commonly designated as gluten. In addition there are, among other constituents, traces of sugar and some diastase. The flour is mixed with water to form a dough; in some types of bread a little sugar is also added. A small portion of the starch is converted into sugar by the action of the diastase. From this point the processes differ somewhat in the dough allowed to ferment spontaneously and that to which leaven of some kind is added.

Spontaneous fermentation is used in the preparation of so-called salt-rising bread and sour dough bread. The latter type is that commonly produced in Germany from the rye flour. In some cases bits of dough from a previous lot are used to facilitate the process. It is probable that the fermentation resulting from this method of preparation is not always the same. In some instances few yeasts are present, the gas production being brought about by organisms of the *Bacterium coli* and *Bact. aërogenes* groups (particularly *Bact. levans* and *Bact. panis fermentati*) which ferment sugar

with development of lactic acid and gas, the latter a mixture of carbon dioxide and hydrogen.

Various bacteria present probably assist in the saccharification of the starch. When sour bread is regularly baked and dough saved from one batch to the next, the yeasts are found to play a much more important part. Alcohol and carbon dioxide are produced, while certain of the bacteria related to *Bacterium lactis acidi* and *Bact. bulgaricum* groups form lactic acid. From 0.2–0.4 per cent of the latter is commonly produced. This process of fermentation results in a bread that is characterized by certain flavors and by a high acid content.

The dough is sometimes inoculated with "homemade yeasts" or barms. These produce a bread resembling in many respects the sour dough bread just described in their high acid content and marked flavor. Some of the flavor is probably also contributed directly by the products of fermentation in the leaven itself. The yeasts and lactic acid bacteria are both present and active in this fermentation.

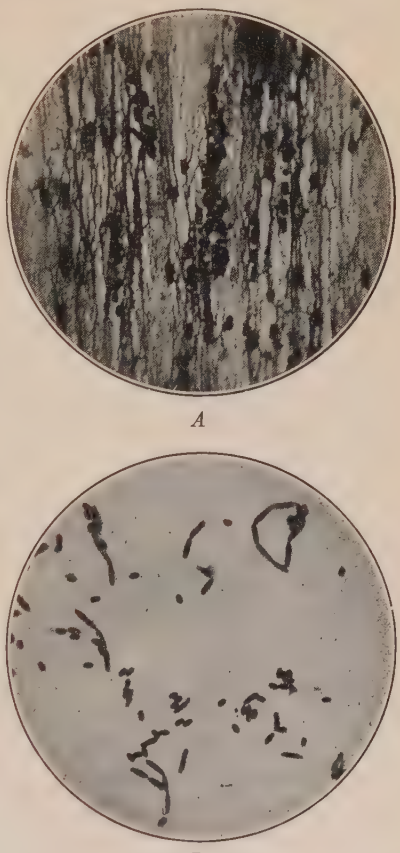
Dough is most commonly inoculated with the relatively pure cultures of yeast now universally sold in the market. The dried yeast cake is frequently soaked in warm water containing sugar to start the yeast into active growth before it is added. Bread prepared from dough raised in this manner has usually less flavor because of the rapid formation of gas due to the heavy inoculation with yeasts and the consequent lack of opportunity for growth of lactic acid and other bacteria. Repeated kneading in the household lengthens the process somewhat and gives more opportunity for development of flavor.

The process of baking destroys most of the organisms present and drives off much of the alcohol formed. The lactic acid is not volatile, and remains.

Abnormal Fermentations of Bread. — The most common abnormal fermentations in bread are (1) undesirably high acidity, (2) ropiness, and (3) "bloody" bread.

Too high acidity may develop as the result of the growth of lactic bacteria for too long a period in the dough before baking, the bread becoming unpalatable as the result of the sourness.

Ropiness ("rope"), "jack in the bread" or slimy bread results from the growth in the bread after baking of certain highly resistant spore-producing bacteria. Several species have been described, unfortunately with such awkward names as *Bacillus mesentericus panis viscosi* and *B. mesentericus fuscus panis viscosi*. They are organisms closely related morphologically to *Bacillus subtilis*. The type of fermentation may occasion considerable trouble in a home, and in a bakery cause serious loss. These organisms seem to be present in most cases in the flour used, possibly in some instances they come from infection of the utensils. They are probably present in most bread, but do not develop and cause rope except when there has been too little development of acid in the leavening process and the bread has been stored at a relatively high temperature. In other words, in order to prevent rapid deterioration of the bread after baking, it is necessary that a certain amount of acid be present in the dough. This is usually formed as a result of the growth of certain lactic acid species. Occasionally the lactic acid is added by bakers. Proper development of the



A

B

FIG. 125. — A bacillus causing slimy bread. *A*, a stained mount prepared directly from slimy bread, showing the bacterial slime, and the sporulating bacilli; *B*, stained mount of the same organism from an agar culture. (× 1000.)

acidity improves the texture of the loaf and renders impossible the development of ropy and stringy bread.

Some of the chromogenic bacteria may form colored spots or areas in the bread. *Serratia marcescens* (frequently termed *Bacillus prodigiosus*) produces a red pigment and is the cause of "bloody" bread.

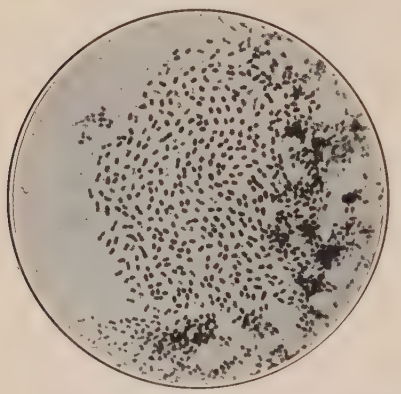


FIG. 126. — *Serratia marcescens*, an organism which causes red spots in bread. (After Günther.)

Modified Alcoholic Fermentations. — The preceding discussion of alcoholic fermentation has been based upon the assumption that the end products of the fermentation are ethyl alcohol and carbon dioxide. This is not strictly true. It was early discovered by students of the problem that other compounds were produced in small quantities. Among these is glycerol ($CH_2OH \cdot CHOH \cdot CH_2OH$). In recent years it has been found possible greatly to increase the proportion of glycerol and decrease the amount of alcohol produced. Such modifications of the course of the fermentation may be accomplished by keeping the reaction of the medium alkaline, or by the addition of sulphites which unite with the intermediate product, acetaldehyde.

CHAPTER XXVI

CHANGES PRODUCED IN NON-NITROGENOUS ORGANIC SUBSTANCES (*Continued*). LACTIC ACID AND PROPIONIC ACID FERMENTATIONS

THE two fermentations to be discussed in this chapter involve the production by microörganisms of two closely related acids, lactic and propionic.

The production of lactic acid from carbohydrates is one of the commonest fermentative changes that occurs in nature. Lactic acid is one of the waste products formed during muscular activity in animals, and it arises as the principal product of the fermentation produced by many organisms. It is usually formed in milk and in fermented foods of several types such as sauerkraut and ensilage.

It is generally assumed that the transformation of sugars into lactic acid is brought about by an intracellular enzyme termed *lactacidase*. Many attempts have been made to isolate such an enzyme from cells, but these efforts have not been completely successful. Lactic acid is alpha-hydroxypropionic acid ($CH_3 . CHOH . COOH$). Inasmuch as the carbon atom in the alpha position is asymmetric, two lactic acids are known, the levolactic and dextrolactic acids, as well as the inactive form which is an equal mixture of the two. The kind of acid formed seems to depend upon the species of organism producing the fermentation, the sugar fermented and the temperature maintained. The transformation of sugar into lactic acid probably proceeds in several steps, but the reaction may be represented as

$$\underset{\text{Dextrose}}{C_6H_{12}O_6} \longrightarrow 2\ \underset{\text{Lactic acid}}{CH_3 . CH(OH) . COOH}$$

As with the production of alcohol by yeasts, so the capacity for the formation of lactic acid from a particular carbohydrate

is dependent upon the species of organism. Some can produce the acid indifferently from many sugars, others require certain of the hexoses, as dextrose, while still others possess no power to form the acid.

Lactic Acid Fermentation of Milk

Milk allowed to stand for a few hours usually undergoes lactic acid fermentation. This accomplishes a certain diminution in the amount of sugar, and curdles or precipitates the casein.

The lactic acid fermentation of milk is brought about almost exclusively by bacteria. These gain entrance to the milk in various ways; in a relatively small proportion of cases they may be present in the milk as it comes from the udder; usually they come from dust or dirt from the surface of the teats, udder, and hair of the cow and from the milk utensils. Because of the extremely rapid development of these organisms when they get into milk, it is difficult to prevent souring. By keeping down the initial contamination as much as possible and by holding the milk at a low temperature, the souring can be greatly retarded.

The bacteria producing lactic acid in milk may be placed in two principal groups, those that bring about little chemical change in the milk other than the production of the acid, and those that cause formation of gas or digestion of protein in addition to the acid formation. The first may be termed the normal or desirable type, the second the undesirable.

The True or Desirable Lactic Acid Bacteria. — The organisms producing lactic acid primarily, belong for the most part to the genera *Streptococcus* and *Lactobacillus*.

The Genus Streptococcus. — There has been much discussion in the literature relating to the souring of milk as to whether lactic acid production in milk is usually due to the presence of streptococci or of short rods occurring in chains. This has given rise to considerable confusion in names. It seems, however, that the organisms frequently alluded to in literature as *Bacillus lactis acidi* or *Bacterium lactis acidi* are for the most part at least, streptococci, and may be included as species of the genus Streptococcus, the most common being *S. lactis*.

Another source of confusion in discussions of the streptococci as lactic-acid producers has been the fact that the first species studied, *Streptococcus pyogenes*, as well as many other species since described, are found associated with diseased conditions in man and animals. This led at one time to recommendations for the condemnation of milk which was found upon microscopic examination to show chains of cocci. It seems now to be well established that several species of non-pathogenic streptococci are the most common organisms instrumental in bringing about lactic acid fermentation of milk.

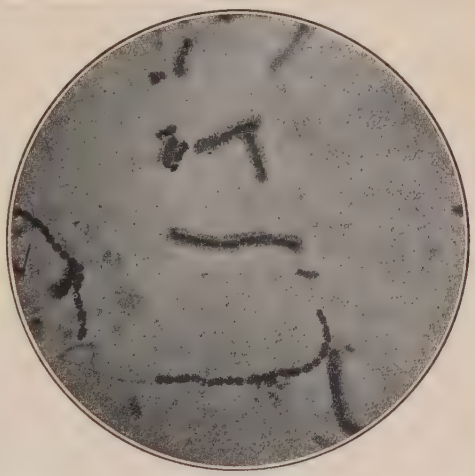


FIG. 127. — Capsulated streptococcus from slimy milk. (× 1000.)

Streptococcus lactis has spherical cells (sometimes slightly elongated), occurring in chains. It is gram positive, does not produce spores and is non-motile. Apparently there are some strains, perhaps distinct varieties, capable of producing capsules. Certain of these capsulated types are responsible occasionally for the development of ropiness in milk, and for the sliminess experienced in certain cultures or starters. *Streptococcus lactis* grows well, but never luxuriantly, on many of the laboratory media. Its growth is greatly increased in amount by the presence of suitable sugars which it may ferment. Media prepared from whey are frequently found to be useful. Lactose agar plates poured from souring milk will show the organisms developing as small pin point colonies, frequently lying somewhat below the surface of the medium. If litmus or some other suitable indicator is present, the medium surrounding the colony will be noted to have become intensely acid. Upon agar slants the colonies are usually more or less separated or

discrete, at first scarcely visible, and at last dew-drop like in appearance. In ordinary broth the organism does not grow well unless sugar is present. Usually the medium clears rapidly by sedimentation. Gelatin is not liquefied.

The common sugars, dextrose, sucrose, and lactose, are all fermented with the production of lactic acid but never with the

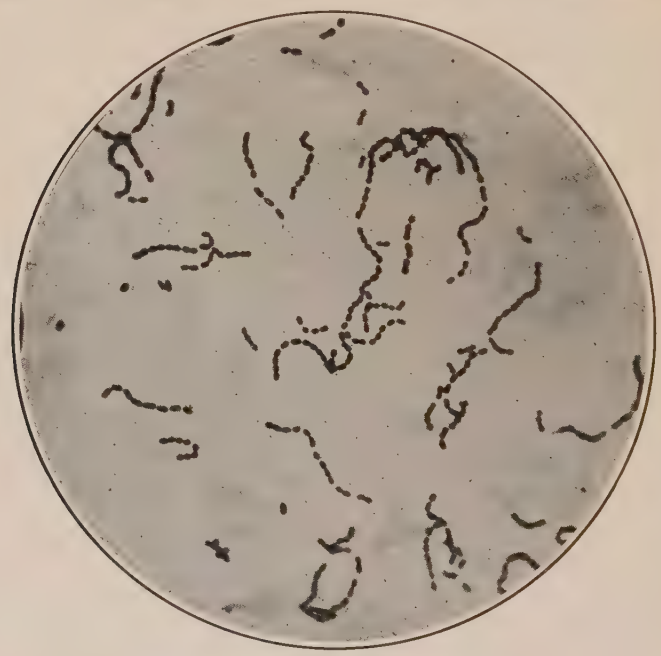


FIG. 128. — *Streptococcus lactis*, from milk. (× 1000.)

formation of gas. There is some confusion in the literature as to the type of acid produced, but pure cultures in certain sugars at least, frequently produce the dextro type only. When grown in milk, sufficient acid is usually formed from the lactose (.5 to 1.25 per cent) so that coagulation or development of an acid curd occurs. The curd so formed is usually smooth, free from gas bubbles, has a pleasant acid flavor and with little or no tendency to shrink and expel the whey. Its optimum growth temperature, apparently, is about blood heat, but it grows well (particularly in milk) at room

temperatures and slowly at the temperature of the ice chest. Apparently there are strains of this organism, perhaps closely related species, which can resist the ordinary temperatures used in pasteurization. This is evidenced by the fact that milk which has been pasteurized in commercial plants usually retains sufficient numbers of these organisms in the living condition so that it sours.

Putrefactive bacteria are practically always present in milk. They rarely develop sufficiently, however, to become prominent because of the much more rapid growth of the lactic acid bacteria and the creation of an acid reaction unfavorable to putrefaction. Opportunity is therefore denied for the production of poisonous putrefactive substances. The complete destruction of all the lactic acid bacteria, as may occasionally occur in pasteurization, or the inhibition of their growth by holding milk for a long period at low temperatures may offer opportunities for the development of these putrefactive forms. It is probable also that the acid production inhibits to some degree the multiplication of pathogenic bacteria.

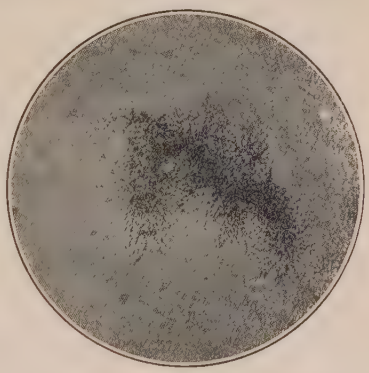


FIG. 129. — Colony of *Lactobacillus bulgaricus* under the low power of the microscope.

Buttermilk owes its flavor and acidity largely to bacteria of this group. Artificial buttermilk is prepared by inoculating sweet milk with a considerable proportion of pure culture of lactic acid bacteria. When the milk has curdled it is beaten or churned so that the casein is divided into fine particles. This is to be preferred to true buttermilk as a beverage because of the greater uniformity in flavor and texture secured. Milk soured in this manner may be flavored with fruit juices and frozen into a kind of milk sherbet. This has been marketed under the name "lacto." The alcoholic beverages produced from milk and discussed in the preceding chapter owe their acidity to organisms of this group.

The ripening of cream preparatory to churning is essentially a souring induced for the purpose of rendering churning easier, and to impart certain desirable flavors and aromas to the butter. Butter may be churned from sweet cream, but such is not salable in most parts of the United States, although in some parts of Europe it is preferred. The bacteria normally present in the cream will usually produce the acid and other products desired, and dependence is placed upon these in the manufacture of butter in the home. In the creamery, however, where the cream comes from many sources and is often old and with objectionable bacteria present, another method is resorted to. The cream is pasteurized, then inoculated with a pure culture of lactic acid bacteria of this group, termed a *starter*. By this means a butter much more uniform in character can be obtained than by relying on the forms initially present in the cream.

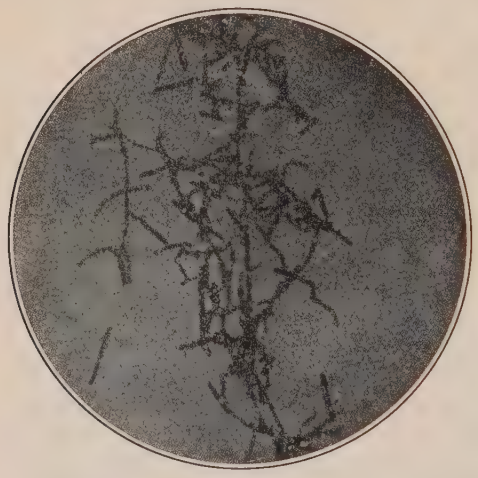


FIG. 130. — *Lactobacillus bulgaricus*, in milk, stained by Gram's method and counter stained with eosin. Gram positive organisms are dark in color, gram negative lighter. (× 1000.)

The flavor and aroma of butter will depend very largely upon the type of organisms which have been growing in the cream. The butter fat absorbs considerable quantities of dissolved substances present in the fermenting cream; it is, therefore, highly desirable that these should be of the proper character. If batches of cream are allowed to ripen spontaneously without the addition of any starter there is apt to be a marked lack in uniformity in the product. A much better and more uniform result is secured by inoculating the cream which is to be ripened with a considerable

amount of desirable lactic acid bacteria. Such organisms are sold under the name of commercial starters. It is generally assumed that they are pure cultures, or practically pure cultures, of *Streptococcus lactis* or some very closely related organism. They are introduced into pasteurized milk and allowed to grow until the milk has reached a satisfactory state of acidity. With this a larger bulk of milk is inoculated and finally the whole mass used as starter in the cream which is to be churned. A heavy inoculation with the desirable microörganisms and their growth products usually overwhelms undesirable types of bacteria and leads to the development of a satisfactory product.

Recent studies have made it evident that the changes brought about in a satisfactory starter are not the result simply of the growth of *Streptococcus lactis*. Another organism closely related to it must ordinarily grow with it. Cream which has been ripened by means of a pure culture of the ordinary *Streptococcus lactis* does not produce butter with as satisfactory a flavor and aroma as that produced by cream which has been inoculated with a mixture of these organisms. The ripening produced by a starter is therefore an excellent example of associative action. The true *Streptococcus lactis* apparently produces very little change in milk or cream other than the development of the pure lactic acid from lactose. The associative organism, also a coccus (either *Streptococcus citrovorus* or *Streptococcus paracitrovorus*), can by its own growth bring about very little change in milk. When grown in the presence of *Streptococcus lactis*, however, the activity of this organism is greatly stimulated and small amounts of volatile acids are developed. It is to the absorption of these and perhaps of other growth products that the butter owes its characteristic aroma and flavor.

Some varieties of *S. lactis*, or closely related species, produce the products which may prove to be undesirable in butter or cream. One of these, *S. lactis* var. *maltigenes*, produces a malty flavor.

The Genus Lactobacillus. — The bacteria of this genus are all gram positive, non-spore-producing rods developing commonly in milk and various fermented food stuffs. A few species have been reported from the alimentary tract of man and animals. The group is sometimes known as the group of *high acid bacteria* because

certain of the species will grow in a medium having a higher hydrogen ion concentration than will permit of the growth of most bacteria, even of the streptococci. This, however, is not true of all of the organisms belonging to the group, for some of them do not produce quantities of acid larger than those developed by *Streptococcus lactis*. Some of the species are relatively short rods. Most of them, however, are decidedly elongated, sometimes almost filamentous. The shortest rod forms apparently intergrade with Streptococcus; it is accordingly sometimes difficult to differentiate between them and *Streptococcus lactis*.

One of the best known species belonging to this group is the *Lactobacillus bulgaricus*. The organism was first described by Metschnikoff. When traveling through southern Russia and the Balkan countries, this investigator was much impressed by the large quantities of fermented milk and sour milk beverages used by the peasant classes as food. He also reached the conclusion that the peasants of this region were particularly long lived. He attributed this longevity to the large use of the soured milk in the diet. He made a study of the favorite milk beverages and succeeded in isolating the organism chiefly responsible for the lactic acid formation. Inasmuch as it was isolated from the Bulgarian soured milk it was given the specific name *L. bulgaricus*. The organism is a relatively large rod, sometimes filamentous. The cells are usually 0.5–1.0 μ in diameter, and 2–3 μ in length. In old cultures, however, some of the rods may be many microns in length. While the organism is definitely gram positive in young cultures, smears made from old cultures may show a mixture of gram positive and gram negative rods. Single cells may sometimes show a portion retaining the gram stain and another portion decolorized and taking the contrast stain. This organism does not grow very readily upon the ordinary culture media, although its development in milk at the right temperature is abundant. The colonies produced in agar resemble a tiny mass of wool. The thread shaped rods will be seen to penetrate the medium in all directions. A stab culture in whey agar or lactose agar gives a fir tree appearance. *Lactobacillus bulgaricus* grows best at temperatures above blood heat, the optimum being 42° to 45° C. Growth

is slow at room temperatures. The total amount of acid formed is usually greater than that produced by the *Streptococcus lactis*, sometimes as much as four per cent being observed. Inasmuch as growth evidently continues in these relatively strong solutions of acid, the organism is termed acid tolerant or *acidophilous*. The curd produced in milk is somewhat slimy, smooth, and does not tend to contract and expel the whey. The organism grows best under anaërobic conditions, better therefore below the surface of the medium than at the top.

Other species which belong to this group of *acid-tolerant* or *aciduric* bacteria have been isolated from the alimentary tract and feces. One of these, *Lactobacillus acidophilus*, has been used rather extensively therapeutically. When ingested with the right food it is able apparently to supplant other organisms in the colon and thereby prevent the growth of undesirable putrefactive bacteria. A related organism found in abundance in the stools of breast fed infants is *Lactobacillus bifidus*.

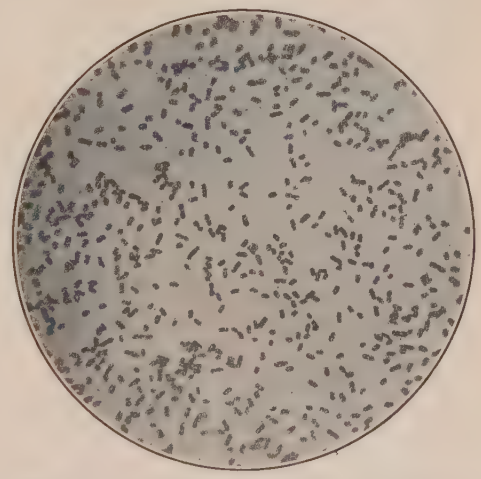


FIG. 131. — *Bacterium aërogenes*. (× 1000.)

Undesirable Lactic Acid Bacteria. — The abnormal and undesirable lactic acid bacteria are represented by a single group, that forming gas in milk.

Gas Producing or Bacterium aërogenes Group. — The organisms belonging to this group are of intestinal and soil origin; *Bact. aërogenes* and *Bact. coli* may serve as examples. They do not differ greatly except in some morphological characters. Both organisms are plump rods, *Bact. coli* motile and without capsules, and *Bact. lactis aërogenes* non-motile and frequently capsulated when grown in milk. They do not produce

spores. They are gram negative, differing in this respect from the other lactic acid bacteria.

The organisms of the group develop readily on ordinary culture media. The colonies on agar or gelatin plates are easily differentiated from those of the preceding group because they are much larger and inclined to be slimy. The optimum growth temperature is about blood heat, but the organisms grow well at lower temperatures. In the presence of suitable carbohydrates these organisms can develop under anaërobic conditions, but when deprived of sugars they are strictly aërobic. Many of the sugars are fermented (among them dextrose, sucrose, usually maltose and lactose) with formation of both acid and gas. The acids formed from the lactose of milk may reach 1 to 1.25 per cent, but are lactic acid only in part, acetic and other volatile acids being also present. In contrast to the normal or desirable lactic acid bacteria, they form the levolactic acid. The gas produced is a mixture of hydrogen and carbon dioxide.

The curd formed in milk by organisms of this group is more or less torn by gas bubbles; it shrinks and expels a considerable proportion of the whey. The flavor developed is quite undesirable. These organisms, particularly *Bacterium coli*, when present in great numbers in milk indicate considerable fecal contamination. Such milk should not be used for human consumption. They also injure milk for the manufacture of cheese and produce undesirable flavors and aromas in butter made from cream soured or ripened by their action.

Lactic Acid Fermentation of Sauerkraut

One of the principal factors in the manufacture and preservation of sauerkraut is the development of lactic acid in quantities sufficient to act as a preservative. The cabbage is cleaned, cut into small pieces, and mixed thoroughly with about 2 per cent of salt as it is packed in a barrel or earthenware vessel. The surface is weighted. The salt dissolves in the sap expressed by the pressure, and by osmosis draws from the cells of the leaves a considerable proportion of their water. The cells of the leaf respire for a time, rapidly utilizing any oxygen present and creating anaërobic condi-

tions. The leaves shrink in size as the water leaves them. The latter soon comes to fill completely all interstices, and constitutes a weak brine having in solution various sugars and nitrogenous materials from the cells, in which are immersed the wilted leaves. This then undergoes a spontaneous lactic acid fermentation, the organisms responsible doubtless originating from the surfaces of the leaves. Probably some of the leaf enzymes also play a part. Usually about 1 per cent of lactic acid is formed. Many other substances are developed, some of them essential to the establishment of the desired flavor in the product.

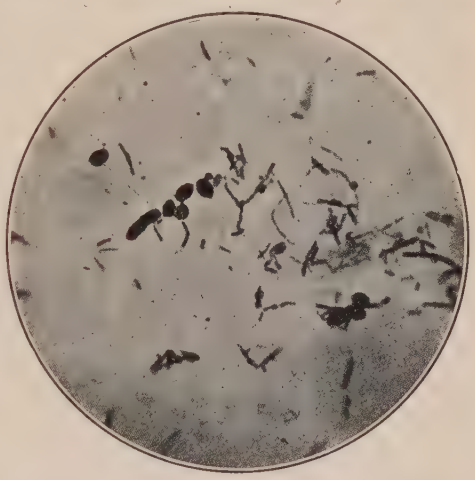


FIG. 132. — Organisms from Sauerkraut. Note the presence of both yeasts and bacilli. (× 1000.)

Many organisms besides the lactic acid bacteria may play a part in this fermentation. Yeasts usually initiate the process by the formation of alcohol and carbon dioxide; this however is soon stopped by the development of the lactic acid bacteria. These are usually of the *Streptococcus lactis* and *Lactobacillus* types, although representatives of both of the other groups have been described. *Oidium lactis* and possibly other molds may grow on the surface of the kraut, and if the material is not kept from the air, gradually oxidize the lactic acid and enable the putrefactive organisms to develop. Occasionally the material fails to undergo normal fermentation, and is spoiled by the growth of the butyric acid bacteria.

LACTIC ACID FERMENTATION OF OTHER FOODS

Many other vegetables, such as green beans, corn, etc., may be fermented and preserved in the same manner as sauerkraut. Dill

pickles are prepared by washing cucumbers, packing them in a cask, and covering with water, with the addition of salt, spices, and sometimes sugar. The soluble sugars of the cucumber cells gradually diffuse out and are fermented with production of lactic acid. This accumulates in sufficient quantities to inhibit the growth of putrefactive bacteria. The lactic acid bacteria present belong to the same groups already discussed in connection with milk. It is uncertain which of these organisms is the most important. The Russians produce "barszcz" (or "borsch"), composed of red beets that have undergone a lactic fermentation.

The preparation of sour dough bread already discussed is based upon lactic acid fermentation by bacteria, probably largely organisms of the *Lactobacillus* group. Ensilage, composed of shredded corn, alfalfa, sorghum, beets, or other similar materials, undergoes a lactic fermentation and owes its preservation largely to the presence of this acid. A certain amount of lactic acid fermentation is sought in the preparation of some beers. This is true in the so-called ginger beer discussed under alcoholic fermentation. Formerly a lactic acid fermentation constituted an early step in the preparation of tomatoes for ketchup.

Propionic Acid Fermentation

A group of bacteria to which the generic name Propionibacterium has been given are known to produce propionic acid ($CH_3 . CH_2 . COOH$) rather than lactic acid from carbohydrates. They are closely related to Lactobacillus, but grow more poorly under aërobic conditions. The fermentative change involved in the production of propionic acid is probably similar to that in lactic fermentation, but the organism has somewhat higher powers of reduction than do the lactic bacteria. The propionic acid bacteria usually produce some gas (carbon-dioxide) and acetic acid as well. They are important in the ripening of certain types of cheese.

CHAPTER XXVII

CHEMICAL CHANGES IN NON-NITROGENOUS COMPOUNDS (*Continued*). ACETIC, BUTYRIC, CITRIC, AND OXALIC ACID FERMENTATIONS

Acetic Fermentation. — Acetic acid is most commonly formed as the result of bacterial oxidation of ethyl alcohol, under aërobic conditions.

$$\underset{\text{Alcohol}}{C_2H_5OH} + O_2 = \underset{\text{Acetic acid}}{CH_3COOH} + H_2O$$

Acetic acid is also formed as one of the products of more or less complex anaërobic fermentations of carbohydrates, but in relatively smaller amounts. Such are termed mixed or hetero-fermentations. Some organisms may produce small quantities of formic and acetic acid coincidentally with alcohol. Such organisms belong to the *Bacterium aërogenes* group of bacteria. The production of acetic acid by propionic bacteria was noted in the preceding chapter. Of the organisms capable of producing acetic acid, the ones of greatest economic importance are the aërobic types that oxidize alcohol. These are the ones that are active in the manufacture of vinegar.

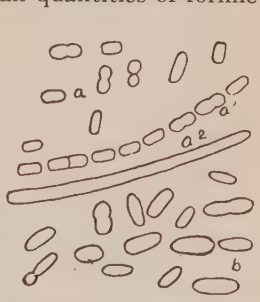


FIG. 133. — *Acetobacter pasteurianum*, one of the acetic-acid bacteria. (Adapted from Henneberg.)

Aërobic Acetic Acid Bacteria. — Many species of aërobic acetic bacteria have been described. They are all closely related, differing in production of acids, fermentative power, and in morphology. The oxidation of alcohol is believed to be due to an intracellular enzyme termed alcohol oxidase, but, like lactacidase, it has never

been separated from the bacterial cells, either because it is destroyed when freed from the sap of the cell or because it is insoluble. Together, these organisms constitute what was originally called *Mycoderma aceti*, or mother of vinegar, which floats on the surface of alcoholic liquids undergoing acetic fermentation. Many species (about fifteen) have been described, all of them bacilli, usually growing in chains, and not producing spores. Some develop only small quantities of acid, and are not used in vinegar manufacture, others form acid rapidly and in considerable quantities. Some are characteristic of acetic fermentation of beer, others are from wine, and still others are important in the so-called quick vinegar process. A few of the most common species will be described briefly.

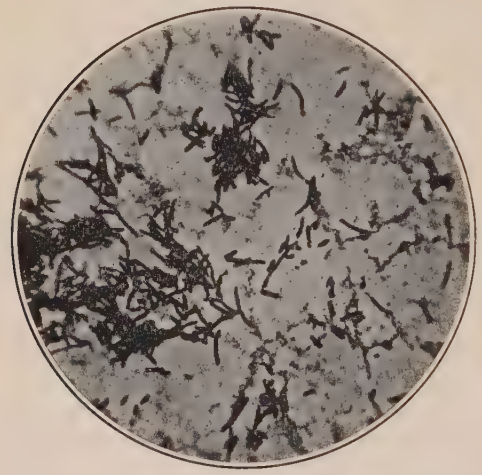


FIG. 134. — Bacteria from mother of vinegar (*Acetobacter aceti*). (× 1000.)

Acetobacter pasteurianum produces a membrane on the surface of souring beer, at first moist, later dryer, marmorate and finely wrinkled. It has also been described as one of the organisms producing wine and cider vinegars. The liquid below the membrane remains clear. The organism grows in chains; occasionally single cells become greatly elongated as filaments. The slimy membrane surrounding the cell wall is peculiar in that it is stained blue by a solution of iodine. This species, as well as some related forms, is noteworthy because of the presence of great numbers of involution forms or hypertrophied cells. They usually consist of greatly swollen filaments. The shape and size of the cells may be varied considerably by the use of different media. The normal

cells are about 1 $\mu \times 2\ \mu$. The organism is non-motile and does not produce spores. It grows readily on the usual culture media, particularly wort gelatin and sugar agar. It produces gluconic acid ($CH_2OH(CHOH)_4COOH$) from dextrose, propionic acid (C_2H_5COOH) from propyl alcohol (C_3H_7OH), and acetic acid (CH_3COOH) from ethyl alcohol (C_2H_5OH). The optimum temperature is about 34° C., the maximum 42° C., and the minimum 5° C. It does not develop in solutions containing more than 9.5 per cent of alcohol, and can produce under favorable conditions about 6 per cent of acetic acid. It is readily destroyed by the heat of pasteurization. Several other closely related species have

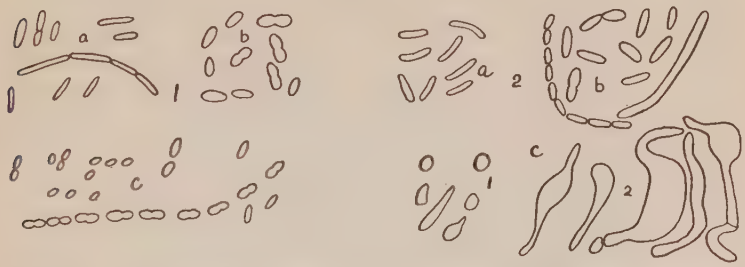


FIG. 135. — 1, *Acetobacter xylinoides* from vinegar. 2, *Acetobacter orleanense*, from vinegar. *a*, *b*, *c*, organisms from various culture media. (Adapted from Henneberg.)

been described from beer vinegar, differing somewhat in morphology and fermentative power.

Acetobacter xylinoides and *Aceto. orleanense* have been described as the common causes of acetic acid fermentation of alcoholic solutions prepared from fruit juices such as wines and cider. Both of these produce a film over the surface of the fermenting medium. Morphologically they resemble *Aceto. pasteurianum*, as they are rods, sometimes occurring in chains, very frequently separated. Involution forms are also relatively common, particularly in *Aceto. orleanense*. They grow readily upon the common laboratory media. They can produce acids from many sugars, but develop best where it is possible to oxidize alcohol to acetic acid and water. In solutions deficient in alcohol but containing acetic acid, they carry the oxidation still further, transforming the acetic acid into

carbon dioxide and water. The cells of *Acet. xylinoides* are 1.2–2 μ $\times$ 0.8 μ. Those of *Acet. orleanense* are somewhat more slender, 1.6–2.4 μ $\times$ 0.3–0.4 μ. The optimum temperature of fermentation lies at about 30° C. The minimum probably is about 10° C.

Acetobacter schützenbachii (with several related species) has been described as the common cause of acetic fermentation in the so-called quick vinegar process. It grows readily upon the ordinary

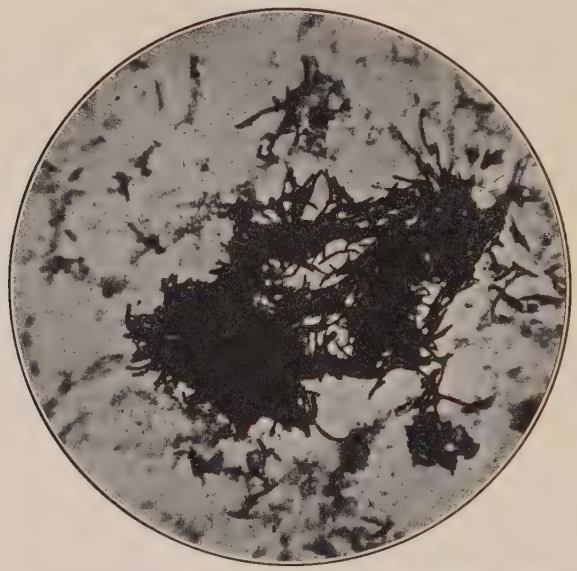


FIG. 136. — Oxidizing bacteria from the surface of vinegar. These organisms oxidize the acetic acid to carbon dioxide and alcohol, thus weakening the vinegar. ($\times$ 1000.)

culture media, but does not develop a coherent film upon the surface of liquids. In this respect it differs from the forms that have been described previously. It is therefore of little significance in the production of vinegar where film formation is an essential. The cells of the organism are usually oval or elongated, not infrequently sickle shaped or irregularly bent with rounded or pointed ends. They are usually in pairs, sometimes united in chains. Considerable differences may often be noted in the size and shape of the cells in the same chain. Involution forms, particularly curved

rods, are not uncommon. These organisms develop in great numbers upon the beechwood shavings used in the quick vinegar process. The organism is relatively slender, 0.3–0.4 $\mu \times$ 3–6 μ. It does not give the cellulose reaction with iodine. It grows best at 25° to 30° C. In the absence of sufficient alcohol, the acetic acid formed may be oxidized to carbon dioxide and water. By the quick vinegar process, in which pure cultures of this organism have been used, acetic acid to the amount of 11.5 per cent has been obtained.

Manufacture of Vinegar. — The raw materials most commonly used for the manufacture of vinegar are wine, cider, and other alcoholic fruit juices, unhopped beerwort, brandy not having too high alcohol content, the fermented juice of the sugar beet, and distilled alcohol in solution. Many other alcoholic solutions, particularly alcoholic beverages, are occasionally used. For the most rapid fermentation it is necessary to have an alcoholic solution containing 5 to 10 volume per cent of alcohol, a temperature of 20–30° C., an abundance of oxygen, and suitable nutrients other than alcohol.

Vinegar as commonly made in small quantities in the household or on the farm is prepared from cider. This is allowed to stand in partially filled barrels or casks until spontaneous souring occurs, or the acetification may be initiated by the addition of " mother of vinegar " (a portion of the film which has developed on the surface of vinegar previously prepared), or by adding a little vinegar of good quality. It is necessary that a considerable surface of the liquid be exposed to the air and that the bunghole remain open to permit aëration. A film forms over the surface of the liquid and the alcohol is changed into acetic acid. This is usually accomplished by organisms of the *Acet. pasteurianum* type. When the fermentation is complete, the vinegar should be drawn off, filtered, and stored in tightly stoppered vessels, as the acetic bacteria and other organisms present otherwise would oxidize the acid gradually and the vinegar would " lose its strength." A portion of the vinegar may be drawn off and the loss made good with fresh cider or wine, using care not to break the film. This added liquid will be rapidly converted into vinegar and the process may be repeated in three or four weeks. In wine-producing countries

wine is commonly used for the making of vinegar. Cider and wine vinegar contain many organic acids, esters, etc., derived from the fruits and resulting from fermentation not found in vinegar from other sources.

Two methods have come into general use in the commercial manufacture of vinegar, each with many local adaptations and modifications. These are the Orleans method and the quick or German method.

The production of vinegar by the Orleans method does not materially differ in essentials from that described above. Wine (or other alcoholic solution) is mixed with good vinegar and placed in a cask sufficiently large to give considerable surface for aëration. Fresh wine is added weekly until the cask is half filled, then a part is drawn off, filtered, and placed in vessels (casks) excluding air. The process of adding wine at intervals is continued, and vinegar is drawn off from time to time. The method was considerably modified by the work of Pasteur. Vats are now employed instead of casks, and a wooden frame or lattice floats on the surface to keep the surface film intact when it has once developed. The rapidity of acetification is directly proportional to the ratio of the area of the surface to the volume of the liquid, hence these vats are shallow. Wine (or cider, etc.) is added daily and an equal amount of vinegar removed.

The rapid or German method had its beginnings in the first half of the eighteenth century in a discovery of Boerhave. He found that vinegar could be produced quickly by allowing wine to trickle through a tall cask very loosely packed with pomace (the residue of the grape after the juice is expressed). This quickly sours, the wine, as it passes down through the cask, is spread out in a thin layer over a considerable area, optimum conditions are thus secured for rapid oxidation, and by the time the liquid has filtered through the mass (or at least after several passages), the alcohol has been changed to acetic acid, the wine to vinegar. Schützenbach in 1823 modified the method. He sought to obtain the maximum opportunity for the liquid to come in contact with the air. His method in all essentials is used extensively to-day. Tall wooden cylinders are partly filled with beechwood shavings that have been thoroughly

washed and inoculated with acetic bacteria by soaking in good vinegar or by pure cultures. Beechwood (sometimes bundles of rattan) is chosen, as it does not impart disagreeable or undesirable flavors to the product. By means of an automatic sprinkling device, the alcoholic solution to be acetified is distributed uniformly over the top and trickles down over the surfaces of the shavings. This exposes the liquid in thin layers over the surfaces. Oxidizing bacteria such as *Acet. orleanense* and *Acet. schützenbachii* here find optimum conditions for development and soon coat the shavings. The alcohol is rapidly converted to acetic acid, and after one or more passages through such an apparatus the liquid becomes vinegar. Air is admitted by openings in the side of the cylinder, the process of oxidation creates sufficient heat so that there is a constant current of air entering these openings and passing upwards. An abundance of oxygen is thus supplied. Wines and similar liquids containing much material in suspension must be clarified before use. Probably distilled alcohol with the addition of fruit juices or chemicals to furnish nutrients other than alcohol for the organisms concerned is most commonly used. Many of the vinegars prepared in this manner are colored by caramel to resemble cider or wine vinegars, but do not contain the acids and extractives of the fruits characteristic of the best vinegar.

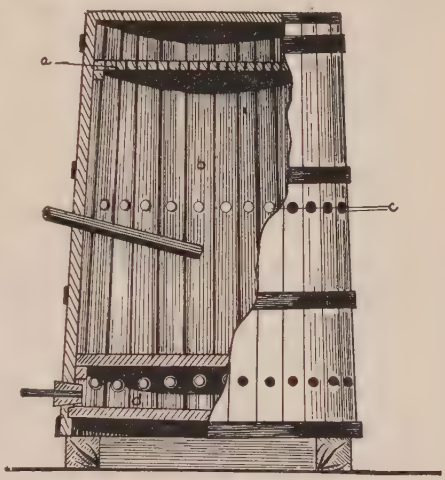


FIG. 137. — Cask for the continuous, quick, or German method of making vinegar. The alcoholic solution trickles through the holes at *a*, and passes over the chips or shavings which fill the space *b*. Air enters through the openings at *c*. The vinegar collects in the lower portion (*d*). (Adapted from Dammer.)

Acetic Fermentation other than in Vinegar. — Many of the lactic fermentations discussed in the preceding chapter are accompanied

by more or less formation of acetic acid. This is particularly true of those brought about by the gas-forming organisms such as *Bact. aërogenes*. Acetic acid is to be found in varying proportions with lactic acid in sauerkraut, dill pickles, silage, and similar fermented foods.

Butyric Fermentation. — The formation of butyric acid is one of the undesirable fermentations encountered in the decomposition of carbohydrates, under anaërobic conditions. This acid is usually only one of the products of the butyric acid bacteria, hence it is difficult to determine with certainty the reactions involved. True butyric acid fermentation may be illustrated as follows:

$$\underset{\text{Dextrose}}{C_6H_{12}O_6} \longrightarrow \underset{\text{Butyric acid}}{C_3H_7COOH} + 2\,CO_2 + 2\,H_2.$$

Because of the powerful and disagreeable odor, this type of fermentation is of economic importance.

The bacteria chiefly responsible for production of butyric acid are closely related, resembling each other morphologically and culturally, and differing chiefly in minor physiological characters. Many species have been described, some of them pathogenic. They are widely distributed in the soil. All are spore-bearing bacilli, usually clostridia, developing only under anaërobic conditions.

Clostridium butyricum, or the closely related *Cl. saccharobutyricum* (*Granulobacter saccharobutyricum* of Beijerinck), may be taken as a type. This organism is present in soil, grains, and decaying matter generally. It is an actively motile bacillus in young cultures, with numerous peritrichous flagella. It stains readily with the ordinary aniline dyes. With certain of these stains, as methylene blue, the cells are decidedly granular; these granules give a blue reaction with iodine and are probably carbohydrate in nature. Spores are produced, usually causing an increase in the diameter of the cell, and the development of a spindle form or clostridium. The cell wall frequently remains around the spore, or may split open at the end. The organism is gram positive. It grows well only under anaërobic conditions in the presence of sugar. The formation of acid, however, soon inhibits its development, and for

maximum growth it is best to add powdered calcium carbonate (chalk) to neutralize the acid as formed. Hydrogen and carbon dioxide are produced in addition to the butyric acid. Smaller amounts of other related acids, as acetic and lactic, may be formed, also butyl alcohol. Gelatin is not liquefied.

The development of butyric acid bacteria in foods usually is inhibited by the lactic or acetic bacteria present. The latter types are easily destroyed by heat, however, while the former, by reason of its resistant spores, may be heated to the temperature of pasteurization or even boiling without being destroyed. For example, dirty milk or milk to which a little soil or garden earth has been added, or macerated potatoes, or grains, may be heated to 80° C. for twenty minutes and incubated for a few days at room temperature (or better at blood heat) when butyric acid fermentation will be evidenced by the evolution of gas and the development of the characteristic odor. It is this property of spore resistance to heat that gives the organism its economic importance. The organisms of this type often cause spoilage of canned goods such as corn and peas. It is extremely difficult in some cases to heat the canned goods to a temperature which will certainly destroy all organisms and at the same time not injure the flavor of the product.

Citric Acid Fermentation. — Citric acid is produced in considerable quantities as a product of the fermentation of sugar. The result of the reaction may be represented as follows:

$$\underset{\text{Dextrose}}{C_6H_{12}O_6} \longrightarrow \begin{array}{l} CH_2COOH \\ | \\ HOC \cdot COOH \\ | \\ CH_2COOH \\ \text{Citric acid} \end{array} + 2\,H_2O.$$

Probably the reaction is in reality more complex than indicated by this formula, possibly the acid is synthesized from some of the decomposition products of the sugar. This seems probable inasmuch as the citric acid has a branched chain, while dextrose does not.

The organisms best known as producers of citric acid are *Citro-*

myces pfefferianus and *C. glaber*, two molds very closely related to Penicillium and Aspergillus. They are not easily differentiated by the unaided eye from the former. They are at first white, then green, and finally brownish gray. Conidiophores are unbranched and bear a cluster of elongated sterigmata at the tip. A chain of conidia is borne at the tip of each sterigma. The genus Citromyces differs from Penicillium in the lack of branching

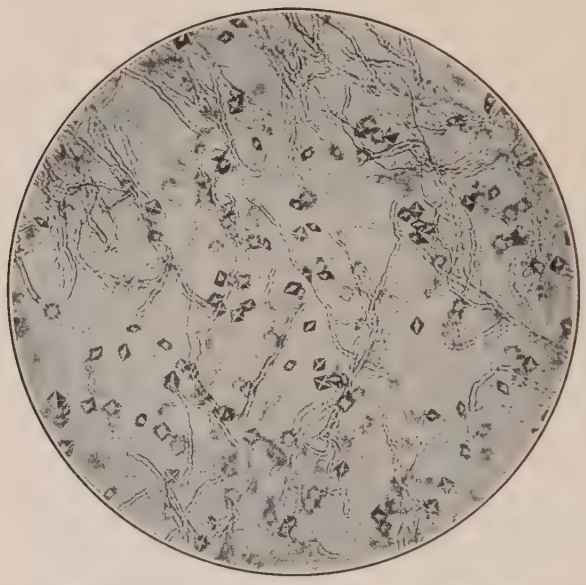


FIG. 138. — Crystals of calcium oxalate formed in beerwort agar by the growth of *Aspergillus niger*. (× 250.)

of the tip of the conidiophore, and from Aspergillus in the fact that the sterigmata all point up and are grouped only on the tip; the latter, too, is little if at all enlarged. These molds have been found in acid fruits. They can produce citric acid from sugar to a concentration of about 8 per cent, and much greater if neutralized by chalk. Citric acid is the principal acid of the lemon and orange and has an extensive commercial use. Attempts have been made to produce citric acid on a commercial scale by the use of these molds. It is difficult to prevent contamination with other

fungi, and the method has failed to compete actively with that acid manufactured from fruits.

Oxalic Acid Fermentation. — Certain molds, particularly of the genus Aspergillus (as *A. niger*), produce appreciable amounts of oxalic acid when growing in a saccharine solution. When the acid as formed is neutralized by the addition of chalk, half the calculated theoretical yield from cane sugar may be obtained. The oxalic acid production by molds has not been utilized commercially, and is of little economic significance.

Other Acid Fermentations. — In addition to the acids discussed, many more have been described among the products of fermentation of carbohydrates. The most important of these are formic, fumaric, and valerianic.

Oxidation of Organic Acids by Microörganisms. — Many microorganisms, principally molds and torulæ, can oxidize organic acids in the presence of an abundant supply of oxygen to carbon dioxide and water. This accounts in large measure for the gradual loss in strength of vinegar, and for the disappearance of lactic acid in sour milk, sauerkraut, etc. By such changes the inhibiting effect of the acid is removed and putrefactive organisms may then develop.

Acetone and Butyl Alcohol Fermentation. — Certain bacteria, particularly species of the genus Aërobacillus, are able to produce considerable amounts of acetone ($(CH_3)_2CO$) in the fermentation of carbohydrates, such as starch. Certain other obligate anaërobic forms of the genus Clostridium are able to produce butyl alcohol (C_4H_9OH) from starch. The commercial production of each of these compounds has developed into industries of considerable commercial significance in recent years.

CHAPTER XXVIII

CHANGES PRODUCED BY MICROÖRGANISMS IN NON-NITROGENOUS ORGANIC SUBSTANCES (*Continued*). TRANSFORMATION OF THE POLYSACCHARIDES, THE RELATED HIGHER ALCOHOLS, AND FATS

Fermentation of Cellulose and Hemicelluloses. — Cellulose $(C_6H_{10}O_5)_n$ is a constituent of most plant cells, and is universal in the higher or green plants as the principal constituent of the cell wall. The changes which it may undergo as a result of fermentation are therefore of considerable importance. It can be broken down into dextrose by heating with an acid. Most of the hemicelluloses when heated with acids break down or hydrolyze into sugars other than dextrose. The hemicellulose of some seeds, such as the date, is changed or hydrolyzed to mannose; the hydrolysis of the hemicellulose from many legumes yields galactose; that of still other seeds and plants, xylose or arabinose.

Many species of bacteria and molds can ferment and utilize the hemicelluloses, fewer forms can utilize the true celluloses. Probably the change in every case consists in an initial hydrolysis of the polysaccharide into its sugar by the enzyme cytase or cellulase. The production of this enzyme by microörganisms may be demonstrated by growing a suitable form on agar containing very finely divided particles of cellulose in suspension. The medium surrounding the colonies of the organism will clear as a result of the secretion of the enzyme and the consequent solution of the cellulose.

The organisms responsible for the decomposition may for convenience be divided into two main groups: the anaërobic forms and the aërobic; the former are bacteria and the latter include both bacteria and molds.

The anaërobic decomposition of cellulose is commonly observed in marshes and stagnant pools, giving rise to the evolution of methane or marsh gas. It may readily be demonstrated in the laboratory by placing a strip of filter paper in a solution containing suitable nutrients and adding a little decomposing horse manure or litter. A slow gaseous fermentation will occur, and the cellulose will gradually go into solution. The organisms bringing about this fermentation have been divided by Omelianski into two types, those that form hydrogen and those that produce methane. Some doubt has recently been cast, however, upon the sufficiency of his investigations and his differentiation of these two types. The organisms are clostridia resembling the butyric acid group of organisms, producing spores and growing in culture media in a manner identical with these forms. The species described by Omelianski, however, do not reveal granules that stain blue with iodine as do the butyric acid bacteria. Acetic and butyric acids are also products of the fermentation.

The aërobic decomposition of cellulose has not been studied very extensively. It is certain that many species of bacteria and a considerable number of species of molds, particularly Penicillia, can decompose cellulose in the presence of oxygen. They are very active in the soil and in heaps of damp straw and manure.

Fermentation of Starch and Inulin. — The fermentation of starch may be simply hydrolytic (a conversion into sugar), or it may be of the butyric acid type. The latter has already been discussed under butyric acid fermentation. Starch is a polysaccharide of the empirical formula $(C_6H_{10}O_5)_x$. It is one of the most important of food constituents, forming the major portion of the carbohydrate diet of man and most animals.

Many molds and bacteria are known to saccharify starch. This is accomplished by the action of the hydrolytic extracellular enzyme diastase (amylase). The starch is first converted into a series of polysaccharides termed *dextrins*, differing from starch in being soluble in water. The dextrins are of several types, probably representing grades of complexity. The one first formed stains red with iodine and is called erythrodextrin. The simpler give no iodine reaction and are called achroödextrins. These

dextrins are then hydrolyzed to maltose.[1] Many organisms carry the change a step farther, to the hydrolysis of maltose to dextrose. The saccharification of starch for the production of alcohol by *Mucor rouxii* and *Aspergillus oryzæ* has already been discussed under the heading of alcoholic fermentation. As a result of this hydrolysis sugars are formed which may be fermented in various ways by a great variety of organisms.

Inulin, sometimes erroneously termed *soluble starch*, has the same empirical formula as starch, but hydrolyzes to levulose instead of dextrose. It is found in some plants. It is fermented by a number of bacteria, and is one of the carbohydrates usually tested to determine the fermentative capacity of a microörganism.

Fermentation of Pectin Substances. — The pectins are compounds related to the celluloses, differing in their reactions and

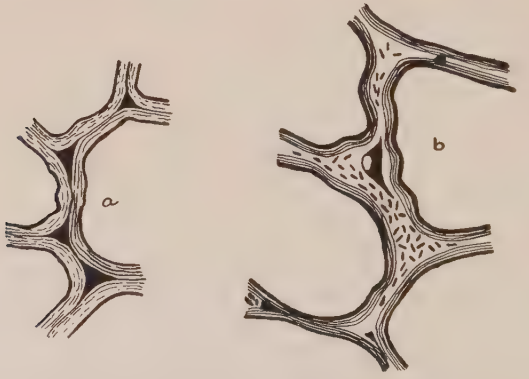


FIG. 139. — *a*, contiguous portions of cells, with middle lamella swollen by the action of the pectinase of bacteria; *b*, a similar section at a later stage, showing the complete dissolution of the middle lamella, and the invasion of the intercellular spaces by the bacteria that secrete the pectinase. (Adapted from Harding and Morse.)

solubilities. Neutral pectoses and pectins are found in the cell walls and the cell sap of many plants. The calcium salt of a related compound, pectic acid, constitutes the binding material between the cells and fibers of higher plants (except where

[1] Some maltose is also formed in the earlier stages of the hydrolysis, along with the dextrin.

modified into wood); it is the middle lamella. A cross section of the stem of any of those plants, when properly stained, will show that the cellulose walls of the cells are not in contact with each other, but are separated by a pectic layer. In some cases it takes very careful observation to make this out; in other cases it is readily demonstrated.

Pectins are even more abundant in the softer plant tissues, such as fruits and certain vegetables.

Many organisms producing disease in plants or decay in plant tissues have been found to secrete an enzyme, pectinase, which hydrolyzes the pectins of the middle lamella, causing them to go into solution and loosening the union of the plant cells or fibers. Several bacteria, for example, have been described which produce so-called soft rots in vegetables, bulbs, etc. As the organism advances the tissues before it soften, owing to the digestive action of the pectinase, and the cells are easily separable from each other. The same enzyme is produced by certain pathogenic and decay-producing fungi. The most important of the practical uses to which this property of microorganisms is put is that of the separation of plant fibers in the process of retting of flax and hemp.

FIG. 140. — *Clostridium pectinovorum*, the organism primarily responsible for the secretion of pectinase and the solution of the middle lamella in the retting of flax. (Adapted from Beijerinck and van Delden.)

Linen fibers are the bast fibers of the flax plant. In cross section of a stem of the flax, these bast fibers are noted as white, many-sided angular cells, with a thick cell wall of cellulose, the cell cavity practically obliterated. These occur in masses, each cell separated from its neighbor by a middle lamella. In longitudinal section the bast cells are found to be long, pointed at the tips, and lying parallel to each other. The production of linen is a problem in separating these bundles of bast fibers from the stems. This is accomplished by the process called retting (literally "rotting"). Two principal methods are used: submergence in water and exposure to moisture, as dew and rain, the so-called water and dew retting processes respectively. In the former the flax is tied into

bundles and thrown into ponds or streams and weighted. Bacteria resembling *Clostridium butyricum* morphologically, but specifically different, have been obtained in pure culture and proved to be capable of producing all the necessary changes. Among those described is *Clostridium pectinovorum* (*Granulobacter pectinovorum*). The changes produced consist primarily of a softening of the middle lamella, a breaking apart of the cells of the plant tissues, and a weakening of the bond between the bundles of bast fibers. The retting must be interrupted at the right stage or the bacteria capable of digesting cellulose will attack the flax fibers and eventually destroy them. These bundles are then dried, and the bast bundles removed and separated from each other by mechanical means. In the land or dew retting process the flax is allowed to lie upon the ground, with the moisture supplied by the dew and rain, until various molds, and possibly bacteria, have destroyed the pectins and permit the removal of the fibers. The retting of hemp is carried out in essentially the same manner as described for the flax, and microörganisms are likewise responsible for the freeing of the fibers. Investigations relative to the utilization in pure cultures of the organisms responsible for retting have been successful, but such methods have not come into common use.

Gum Fermentations. — This topic may be considered under two headings: the transformation of vegetable gums by organisms and the synthesis of gums by organisms.

Transformation of Gums. — Gums are compounds closely related to cellulose and the hemicelluloses; all swell considerably in water, some go into solution, and when heated with acids, they hydrolyze into simple carbohydrates (mostly reducing sugars) and organic acids. Gum arabic, for example, is decomposed into arabinose and galactose, cherry gum into arabinose, gum tragacanth to arabinose, xylose, and fucose. These gums are not easily decomposed by microörganisms, but in nature they slowly undergo decomposition in much the same manner as cellulose. Several organisms have been described as capable of digesting gelose, the principal constituent of the unusually resistant agar-agar prepared from certain seaweeds, and extensively used as an inert material for the solidification of media.

Synthesis of Gums and Gumlike Materials. — Many bacteria and a few molds and yeasts, when growing under suitable cultural conditions, produce considerable quantities of gums and gumlike materials. Some of these, as mucin, are nitrogenous in nature and will be considered later. Most, however, are polysaccharides that break down into reducing sugars when heated with acids. In some cases they are pectins.

It is probable that these materials are produced by the formation of thick capsules or sheaths composed of gums. These swell and partially dissolve in the nutrient medium. The gums and slimes thus produced are of considerable economic importance.

Gummy or slimy fermentation has frequently been a source of trouble in the manufacture of cane sugar. The organism responsible is the *Leuconostoc mesenteroides* which produces gummy masses, sometimes of considerable size. The organism grows readily in ordinary media, but does not produce a capsule. In solutions of sugars, particularly cane sugar, however, it forms thick capsules which fuse into a gelatinous mass. This gum of the capsule has the empirical formula $(C_6H_{10}O_5)_n$, and when heated with acids, it is hydrolyzed to dextrose. It is therefore a dextran.

Wine sometimes undergoes a viscous fermentation, being changed to an oily liquid that can be drawn out into filaments. Micrococci such as *Micrococcus viscosus* have been described as the cause.

Beerwort and beer not infrequently undergo slimy fermentation. This is particularly true where pure culture methods are not utilized in inoculation. A number of bacteria have been described as capable of bringing about this change. Probably the most important are *Bacillus viscosus* and *Sarcina viscosa.*

The juice of the sugar beet used in the production of sucrose sometimes undergoes the gelatinous fermentation due to the *Bacterium pediculatum.* This organism differs from most of the other slime-producing organisms in that the secretion is not uniform over the whole surface of the body. In stained mounts the organism appears mounted upon stalks of gelatin.

Various plant infusions, unless sterilized or kept from fermenting by the addition of preservatives, not infrequently undergo gummy fermentation. This is true of infusions of digitalis, senega, extracts

of various other herbs, and sugary solutions of all kinds. These fermentations are particularly troublesome to the pharmacist.

Slimy fermentation of milk has been described by many investigators, and several distinct species of bacteria are known to bring it about. It is believed that some representatives of practically all groups of bacteria found developing in milk are capable of producing these slimes or gums. Some of them have been described as producing mucin. Most of them, however, produce galactans or dextrans. *Streptococcus hollandicus* is utilized for the production of "*lang Wei*" or ropy whey and milk, the latter being used in the preparation of Edam cheese. Similar organisms have been described from the Scandinavian "*lang Milch.*" *Bacterium lactis viscosum* is probably a variety of *Bacterium aërogenes* capable of producing an unusual amount of slime. Other organisms, micrococci, streptococci, and bacilli, have also been described. The ability to produce capsules is somewhat evanescent as a culture seemingly without reason may acquire or lose this property. It is sometimes found in creamery practice that the pure culture used as starter after transfer to the milk may acquire the power of forming slime. It sometimes happens that milk from the udder of one particular cow in a herd will contain these slime-producing organisms and the milk in consequence from that herd will become slimy under favorable conditions. In other cases sufficient care is not used in the cleaning and sterilization of the milking utensils; in still other cases the water supply used has been found to be involved. Some of the bacteria which produce slime in milk also produce considerable quantities of acid, others are gas producers, others seem to be more or less inert, and still others bring about the development of decidedly disagreeable flavors. The ropy milk of Holland and the Scandinavian countries already mentioned is commonly used as a beverage. The peculiar flavor is probably not altogether due to the slime-producing organism, but to the associative action of several species.

Altogether about thirty species of bacteria have been described as capable of producing ropy or slimy milk. Of these six species produce more or less pigment. One form (*Bacterium viscofucatum*) produces a bluish green color in milk at room temperatures. Three

species, a coccus and two bacilli, produce a red pigment, and two bacilli produce yellow or orange. Two species only in the whole group produce gas in milk. Six species of bacilli and two of cocci have been described as developing the viscosity, in large part, at least, as the result of proteolytic changes. Five species of cocci and eleven of rods do not digest the curd. One species in particular, the *Bacterium visco-symbioticum*, is of interest because it produces viscosity only when growing in mixed cultures with *Streptococcus lactis*, an excellent example of an associative action. Ten species develop sufficient acid to cause coagulation of the milk.

The theory has been advanced by Grieg-Smith and others within recent years, that many of the gums supposed to be physiological secretions of plants, such as the gums produced on certain acacias, gum tragacanth produced on a leguminous plant (Astragalus), gum arabic, etc., are in reality due to the growth of certain species of bacteria in the sap which exudes from wounds in these plants. Pure cultures of some of these organisms have been found to produce very similar gums when grown in suitable nutrient media in the laboratory.

Hydrolysis of Disaccharides. — The disaccharides, lactose, sucrose, and maltose, all having the empirical formula $C_{12}H_{22}O_{11}$, are hydrolyzed by many microörganisms into the hexose monosaccharides. In some cases the transformation is brought about by a specific enzyme. Organisms producing the enzyme maltase transform maltose into two molecules of dextrose according to the reaction.

$$\underset{\text{Maltose}}{C_{12}H_{22}O_{11}} + H_2O \rightleftharpoons 2\ \underset{\text{Dextrose}}{C_6H_{12}O_6}.$$

Maltase has been described from many yeasts, molds, and bacteria. Probably any organism which can bring about fermentation in maltose secretes this maltase and then directly ferments the dextrose so obtained. Many organisms likewise produce sucrase or invertase, transforming sucrose into dextrose and levulose. Fewer organisms can ferment lactose inasmuch as there are comparatively few that produce lactase. This is particularly true among yeasts.

Fermentation of Fats. — Fats are glycerides of fatty acids. The general formula is $C_3H_5 . R_3$, where R represents a fatty acid radical. The three commonest of the fats are palmitin ($C_3H_5(C_{15}H_{31}COO)_3$), stearin ($C_3H_5(C_{17}H_{35}COO)_3$), and olein ($C_3H_5(C_{17}H_{33}COO)_3$). Three stages or types of changes are to be considered with reference to the fermentation of fats: the hydrolysis of fats into glycerol and fatty acids, the fermentation of the glycerol, and the fermentation of the fatty acids thus formed.

Using stearin as an example, we may represent the hydrolysis of fats in the following manner.

$$\underset{\text{Stearin}}{C_3H_5(C_{17}H_{35}COO)_3} + 3\ H_2O = \underset{\text{Glycerol}}{C_3H_5(OH)_3} + 3\ \underset{\text{Stearic acid}}{C_{17}H_{35}COOH}.$$

This change is produced by the fat-digesting or lipolytic enzyme, *lipase.* The enzyme is formed by many microörganisms, principally bacteria and molds. It is evident that a pure fat free from water cannot undergo this change. The ease with which fats can be largely freed from water explains the fact that they can be preserved as food for a considerable period of time. In the presence of moisture, however, and particularly in the presence of other organic matter, fats are actively hydrolyzed by many organisms. Kruse lists eighteen species capable of producing this change. They are important as the common cause of spoiling of many fat foods, although it is probable that in most cases this simple hydrolytic cleavage is not directly responsible for the sum total of rancidity developed or for disagreeable odors and flavors. The specific organisms may be considered under the next heading.

Fermentation of Glycerol. — The glycerol formed as one of the products of hydrolysis of fats undergoes various changes as a result of the activity of microörganisms. *Bacterium coli* among others converts it into alcohol and formic acid.

$$\underset{\text{Glycerol}}{C_3H_5(OH)_3} \longrightarrow \underset{\text{Alcohol}}{C_2H_5OH} + \underset{\text{Formic acid}}{HCOOH}.$$

Bacterium orthobutylicum produces butyric acid, carbon dioxide, and hydrogen. Possibly the reaction bringing about this change may be represented as follows:

$$2\,C_3H_5(OH)_3 \longrightarrow C_3H_7COOH + 2\,CO_2 + 2\,H_2,$$

Glycerol — Butyric acid

although the real changes involved are undoubtedly much more complex. Many other species of butyric acid bacteria are capable of producing this acid from glycerol. Inasmuch as they are very commonly present in the soil and produce resistant spores, they ordinarily withstand the process of pasteurization and are not uncommon in milk and in butter. It is probably the development of butyric acid in this fashion that is responsible, in part at least, for the development of rancidity in butter. The organisms may develop within the water globules of the butter. These droplets contain small quantities of milk sugar, and proteins, which enable bacteria to start growth under favorable conditions, and by means of the hydrolyzing enzymes or lipase they produce, cause the freeing of glycerol, which they then ferment with the production of butyric acid and related compounds. Butter fat normally contains a small percentage of butyrin, and the hydrolysis of this fat is responsible for the liberation of some of the butyric acid. The use of salt in the preparation of butter insures that the water globules shall be practically saturated solutions of sodium chloride. Under these conditions the hydrolytic bacteria do not develop rapidly. The salt, in other words, acts as a preservative.

Fermentation of Fatty Acid. — The fatty acids produced as a result of hydrolysis of the fats may also be changed by certain microörganisms. Very few species have been described, however, which are capable of transforming these compounds. The salts of the simpler fatty acids, such as formic, acetic, propionic, valerianic, and butyric, can be utilized by many species of molds and a few bacteria; usually, however, only in the presence of oxygen.

Fermentation of Paraffins. — Organisms have been described which can oxidize paraffins and related compounds. These changes take place very slowly, as the paraffins are among the most resistant of the organic compounds known.

CHAPTER XXIX

CHANGES PRODUCED BY ORGANISMS IN ORGANIC NITROGENOUS COMPOUNDS. GENERAL DISCUSSION

The proteins and related compounds are complex organic substances containing carbon, hydrogen, oxygen, nitrogen, usually sulphur, and frequently phosphorus, or iron. The molecules of ordinary proteins are of great size. All are alike in that by a series of hydrolytic cleavages they may be converted into simpler and simpler compounds, the *essential* ultimate products in every case being alpha-amino acids. This fact in the hands of Emil Fischer and his associates has led to a fair understanding of the manner in which proteins are constituted. The amino acids can be caused to combine; for example, two molecules of glycocoll, $CH_2 . (NH_2) . COOH$, can, by a series of transformations, be caused ultimately to unite, with the elimination of water. Disregarding the intermediate products the process may be represented as follows:

$$\underset{\text{Glycocoll}}{NH_2CH_2COOH} + NH_2CH_2COOH \longrightarrow \underset{\text{Glycyl glycine}}{NH_2CH_2CONHCH_2COOH} + H_2O.$$

Such a compound is termed a *peptid*.[1] In a similar manner other amino acids may be caused to combine to form peptids. It will be noted that the peptids are also amino acids; hence they, too, can combine to form more complex compounds, termed *polypeptids*. A polypeptid has been formed synthetically by the union in the manner indicated above of eighteen molecules of amino acids, having the molecular weight of 1213. There is probably no real

[1] If the peptid contains two amino-acid radicals, it is called a dipeptid; if three, a tripeptid, etc.

distinction to be drawn between a polypeptid and a peptone. Compounds more complex than polypeptids have thus far not been synthesized. Decomposition of proteins shows that by the reverse process of hydrolysis they are broken down into these same products. We can then conceive of the protein molecule as being built of constituent amino acids (sometimes called the " building stones," " *Bausteine*," of the protein), these united into groups called peptids, and these peptids into still larger groups called peptones, and these in turn into proteins.

The decomposition of proteins by bacteria as well as by chemical means is to be considered as a successive breaking up of the protein into compounds having a lower and lower molecular weight, until the amino acids are reached. By many organisms the transformation is carried still further, and the nitrogen appears as ammonia. In addition to these hydrolytic cleavage products of the growth of organisms, however, there are many nitrogenous compounds formed that are in part synthetic. It is frequently difficult to differentiate these from the true analytic products.

Microörganisms may be classed in three groups, using their relationships to proteins as a basis. There are some organisms wholly unable to utilize proteins as foods; some of these can utilize peptones or amino acids, but some can secure nitrogen for development only from inorganic compounds. A second group may be able to grow upon proteins, but do not bring about any digestion or profound modification in it. Such are many of the pathogenic bacteria, among them the organisms causing pneumonia, tuberculosis, and diphtheria. The third group includes those organisms that take an active part in the breaking down of organic nitrogenous matter in nature. Some of these liquefy solid or coagulated proteins, rendering them soluble by conversion into proteoses and peptones. This process may be termed *proteolysis* (liquefaction or digestion of proteins) or *peptonization* (formation of peptones). The same or other organisms may transform the peptones into amino acids and these into ammonia.

Solid or colloidal proteins cannot serve directly for the nutrition of microörganisms. For this purpose they must be transformed into soluble compounds capable of diffusing through plant mem-

branes, particularly the ectoplast of the cell. This change must come about as the result of the activity of extracellular enzymes. But living cells go much farther and bring about the conditions usually described as putrefaction. This consists in the development of many compounds, frequently odorous, including ammonia and amines, carbon dioxide, acids of the aliphatic and aromatic series, hydrogen sulphide, mercaptans, methane, phenol, skatol, indol, and others.

Organisms important in bringing about decomposition of proteins may be divided (Kruse) into four distinct groups: the strict anaërobes, the facultative anaërobes, the aërobic bacteria, and certain molds. These will be considered in turn, the principal organisms of each group being described together with the most important compounds formed as a result of the decompositions they bring about.

Anaërobic Putrefactive Group of Bacteria

True putrefaction, that is, the disintegration of proteins with the production of various foul-smelling compounds, is generally ascribed to a group of anaërobic bacteria. Among them are certain disease-producing forms, such as those causing blackleg in cattle (*Clostridium chauvœi*) and malignant œdema (*Cl. œdematis*). These are of relatively little importance, of course, as actual producers of putrefaction. The list of saprophytic forms is now a long one, but they seem to be for the most part closely related. *Cl. putrificum* and *Cl. welchii* are among the most common and important.

Clostridium putrificum is common in laboratory dust and in the feces. There is good reason for believing it (or some one of the closely related species) to be the commonest cause of putrefaction of meat and other proteins. It is a slender rod with rounded ends, 5 μ–6 μ in length, single or in chains. Spores are produced at the ends of the rods. They are greater in diameter than the mother cell, and give a drumstick appearance to the rod. The organism grows readily in artificial media. It may be isolated by inoculating a mixture of egg white and water with soil, cheese, or fecal material and heating to 80° for ten minutes. This destroys all non-spore-forming organisms. *Cl. putrificum* can grow in this

medium; most others cannot develop in native proteins. An intense putrefactive odor soon appears, due to the development of hydrogen sulphide (H_2S) and methyl mercaptan (CH_3SH). The solid protein is liquefied, peptones, amino acids, and related compounds being formed. The butyric acid and ammonia developed also contribute to the odor. It should be emphasized that this organism is the type of the few bacteria that can decompose native proteins such as egg albumin, fibrin, serum albumin, etc., under anaërobic conditions and in the total absence of carbohydrates. It is the putrefactive bacillus *par excellence*. It is believed to be of importance in the intestines, particularly the colon, under certain diseased or abnormal conditions, as the cause of intestinal putrefaction.

Clostridium welchii may be taken as a representative of a considerable group of organisms that can cause active decomposition of proteins in the presence of carbohydrates. It is common in soil and has been repeatedly isolated from excreta. It is a large rod, $1\ \mu \times 3$–$6\ \mu$, single or in short chains. It is non-motile (most of the related forms are motile). Spores are not readily produced except on the surface of blood serum under anaërobic conditions. This is not true of all related forms, as some develop spores abundantly in culture media. The spores are equatorial in position and the cells become swollen or clostridia-like. Capsules may sometimes be observed. It is gram positive. It may be isolated from soil by inoculating sterile milk, covered with paraffin oil to exclude the air, with an infusion of rich soil followed by heating to 80° C. This does not destroy the spores of this organism, but will kill the usual lactic acid forms. It should be incubated at blood heat. In twenty-four hours the milk will be found to be coagulated and developing gas. The casein is digested. Organisms of this type are probably quite as common and important in the decomposition of proteins as is the *Cl. putrificum*. Carbon dioxide and hydrogen are formed most abundantly from sugar, but are also produced though more slowly from proteins. Butyric acid, hydrogen sulphide, mercaptans, indol, and other foul-smelling compounds may be formed. Gaseous anaërobic putrefaction may be generally regarded as brought about by this organism or a closely related species.

Group of Facultative Anaërobic Proteolytic Bacteria

This is commonly designated as the proteus group of bacteria, due to the proteus-like or amœboid character of the colonies which these organisms form on gelatin. Several members of the group have been described, the most important being *Proteus vulgaris* and *Proteus mirabilis*. The first may be regarded as typical of the group. All are facultative anaërobes causing active decomposition of proteins.

Proteus vulgaris has been repeatedly isolated from decaying meat. It is a motile bacillus $0.6 \times 1.2\text{–}4\ \mu$. It stains readily, is gram negative, and does *not* form spores. It grows readily on culture media. On agar and gelatin it forms thin, irregular colonies; liquefaction occurs in the latter medium. Development is best at room temperature. It produces acid and gas from dextrose and sucrose, but not from lactose. Casein is liquefied, but it is somewhat doubtful whether this organism can digest such proteins as fibrin and egg albumin under anaërobic conditions. Indol and other malodorous compounds may be formed in sugar-free media. The products of the decomposition of proteins by this organism have probably been studied more than any other. Not all of the data recorded, however, are dependable, for it is by no means certain that some of the strict putrefactive anaërobes were excluded in all cases. This organism is believed by some authorities to be an occasional cause of meat poisoning.

Aërobic Proteolytic Bacteria

Certain of the spirilla are able to decompose some native proteins and related compounds such as gelatin. Among these is the Asiatic cholera organism. Such forms probably do not play any very important part in nature in the decomposition of nitrogenous compounds. Far more important is the *B. subtilis* or hay bacillus group of bacilli. These organisms are all able to decompose proteins under aërobic conditions, frequently without the development of disagreeable odors characteristic of putrefaction. The principal representatives of the group are *Bacillus subtilis* or the hay bacillus, *B. mycoides* or the root bacillus, and *B. vulgatus* or the

potato bacillus. Many other species have been described. They are everywhere abundant in the surface soil, and probably constitute the most important group of decay-producing organisms in nature. They are particularly active in the production of ammonia. *B. mycoides* may be used to illustrate the characters of the hay bacillus group.

Bacillus mycoides is a large rod, usually occurring in long chains, and commonly with truncated ends. It is very sluggishly motile in young cultures, nonmotile in older cultures. It produces spores readily. These are equatorial in position and do not usually cause a marked enlargement of the cell. The cells stain readily and are gram positive. The organism grows well upon culture media. The colonies on agar are quite characteristic. They are exceedingly irregular and spreading, and when examined under the low power of the microscope, are found to consist, at least on the margins, of masses of long filaments like curled hair. In gelatin stabs these filaments radiate from the line of stab, giving a characteristic inverted fir-tree appearance. The gelatin is liquefied. Milk is first coagulated by a rennetlike enzyme and the casein afterwards digested. Blood serum and some other proteins are also liquefied. Peptones and amino acids are formed, eventually a large proportion of the nitrogen appearing as ammonia. Occasionally aromatic compounds such as skatol and indol may be formed, as also some of the fatty acids, such as acetic, butyric, and valerianic.

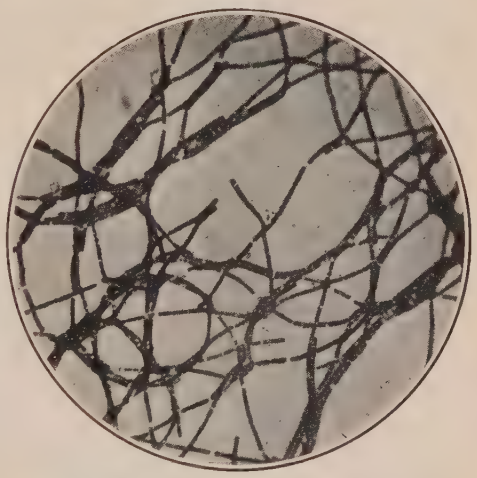


FIG. 141. — *Bacillus mycoides*. Note long chains of truncate rods. Some of the cells show incipient spore formation. (× 1000.)

The importance of these organisms in nature in decomposing nitrogenous compounds with the formation of ammonia can scarcely be overestimated. They are essential in agriculture for the maintenance of soil fertility.

Another group of aërobic proteolytic bacteria of considerable importance is that of the green fluorescent bacilli. The chief

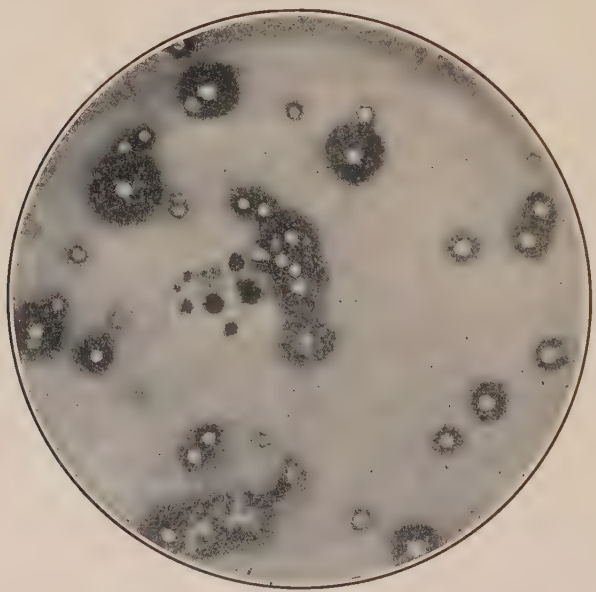


FIG. 142. — Plate culture of a proteolytic organism and a non-proteolytic form growing on casein agar. The large colonies are surrounded by a clear area resulting from the proteolysis of the casein, while the small colonies of the non-proteolytic form are visible only in these clear areas.

representative of this group is *Pseudomonas fluorescens*. This is an organism not uncommon in water, sometimes present in soils. It is an actively motile rod with polar flagella, stains readily, is gram negative, and does not produce spores. When grown on suitable nutrient media, a green fluorescent pigment is diffused through the medium. Gelatin is liquefied, and other related compounds digested. A large proportion of ammonia is produced, as well as several other compounds.

Molds Producing Proteolysis

A considerable number of molds have been described that can proteolyze native proteins and related compounds. Among these are species of Penicillium, Aspergillus, Cephalothecium, and Mucor. A long series of degradation products resulting from their activity have been described. Ammonia is developed in considerable quantities by some forms. At least two species of Penicillia will be noted as of considerable importance in the ripening of cheeses.

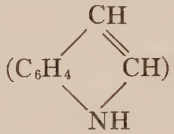
Yeasts also produce autolytic proteolytic enzymes that have been much studied. They are not generally active, however, in producing proteolysis in nature.

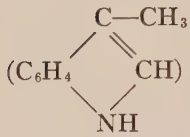
Economic Importance of Organisms Acting on Proteins. — The organisms acting on protein matter are of economic importance from several distinct points of view. That organic nitrogenous compounds must be broken down in nature into ammonia and nitrates that they may serve as nutrients to vegetation is evident. These organisms likewise may interfere with the preservation of food by changing its consistency, its flavor or odor, or even by producing definite poisonous decomposition products termed *ptomaïnes*. The changes in consistency are of particular importance with certain food products, such as cheese, and will be discussed under the general head of ripening processes. The same may be said of the development of desirable flavors. However, the development of undesirable flavors and odors and of ptomaïnes calls for brief discussion.

Putrefactive Substances Having Disagreeable Odors and Flavors. — A complete discussion of the compounds capable of affecting the flavor or aroma that may be formed in nitrogenous food would include a discussion of practically all decomposition products formed. A few distinctive products among those most commonly found will be noted.

Butyric acid (C_3H_7COOH) and acetic acid (CH_3COOH) are not uncommonly formed in protein decomposition. In the putrefaction of such foods as meats, it is possible that these may originate from the fats or carbohydrates present rather than from the

proteins. Some putrefactive bacteria, however, do form butyric acid from the native proteins.

Indol  and methyl indol or skatol

 are commonly formed from native proteins

or peptones in the absence of carbohydrates by many organisms. These have an intensely disagreeable fecal odor. They are developed from the amino-acid tryptophane.

Among the most disagreeable of the odors developed is that from the mercaptans, particularly methyl mercaptan (CH_3SH). These and certain related compounds containing sulphur are the common causes of the worst odors attendant upon putrefaction. Hydrogen sulphide is also commonly formed, as in rotten eggs.

Ammonia is sometimes evolved in sufficient quantities to be distinctly noticeable. Some of the compounds listed among the ptomaïnes are also malodorous, as trimethylamine ($(CH_3)_3N$), often detectable in the brine used for preserving herrings.

Ptomaïnes. — Certain microörganisms when growing under anaërobic conditions may attack amino acids, eliminating carbon dioxide, thereby changing the compound from an amino acid to an amine. This process is termed *decarboxylation*. It may be illustrated by the following reaction:

$$\underset{\text{Alanine}}{CH_3CHNH_2COOH} \longrightarrow \underset{\text{Ethylamine}}{CH_3CH_2NH_2} + CO_2.$$

Amines produced as a result of the action of microörganisms are frequently termed *ptomaïnes*. Some of the amines thus developed are malodorous. A few are distinctly poisonous. For example, the histamine formed by the decarboxylation of the amino acid called histidine is extremely poisonous. Among the ptomaïnes (amines) most commonly produced by microörganisms is *tri-*

methylamine $(CH_3)_3N$. This gives a characteristic odor to herring brine and decaying fish. It is produced in pure cultures by certain bacteria. It is not highly poisonous. Other ptomaïnes which have been isolated are *putrescine* (tetramethylenediamin $NH_2C_4H_8NH_2$), *cadaverine* (pentamethylenediamine $NH_2C_5H_{10}NH_2$), and *neurine* $(CH_3)_3C_2H_3NOH$. The latter is very poisonous. It should be noted that apparently food poisoning is but rarely due to the presence of ptomaïnes. Most of the cases of ptomaïne poisoning which have been recorded in the literature or which are popularly supposed to be of this type are in reality either infections with specific bacteria or are the result of eating foods containing true toxins or endotoxins produced by the specific bacteria, and are not primarily the result of a decomposed protein.

CHAPTER XXX

RELATIONSHIP OF MICROÖRGANISMS TO CHANGES IN CERTAIN PROTEIN FOODS

Ripening of Meats. — The flesh of animals is commonly subjected to a more or less protracted ripening process before it is used as food. With the changes that occur during this process microörganisms normally have little or nothing to do. By the time the muscle tissue has cooled, or even before, the condition known as *rigor mortis* sets in. This is a stiffening of the tissues due to two factors; in part to the more or less complete solidification of the fat, but principally to the coagulation of the muscle protein, that is, the transformation of the myosinogen into myosin. Authorities are not entirely agreed upon the cause of the latter change. Lactic and some other acids are formed in the muscles, probably as a result of the continued metabolism of the cells after the death of the animal. An enzyme, one of those to be classed as autolytic, seems to be freed upon the death of the cell. It is related in action to the lab ferment or rennin. The combined action of acid and enzyme is to coagulate the muscle protein. The flesh thereafter gradually becomes more tender, possibly more digestible. This is probably due to the continued action of the proteolytic autolytic enzymes of the cells. This may be demonstrated experimentally by the removal of a portion of tissue, or an entire organ, such as a liver or spleen, under aseptic precautions to prevent bacterial infection and activity. When kept in physiological salt solution, such a tissue undergoes autolysis, and in course of time becomes more or less completely digested. Such an autolysis, only partial, is the essential transformation in the ripening of the flesh foods. In a certain degree, this seems to be desirable. It is sometimes difficult, however, to differentiate between this

normal change and putrefactive changes brought about by micro-organisms.

Putrefaction of meat is brought about by the organisms already discussed in the preceding chapter. It is not probable that any of these play any important part in the normal ripening.

Ripening of Cheeses. — Cheeses are divided into two classes, those in which the curd is formed by the action of lactic acid and those in which it is developed by the action of rennet. These may be discussed separately.

Acid Curd Cheeses. — Milk, usually whole milk, is allowed to sour spontaneously or as a result of inoculation with starter. The curd is separated from the whey by heating, and formed into balls or cakes. In some cases, it is mixed with cream or butter before use. These constitute the so-called cottage cheeses and Dutch cheeses commonly prepared in the household. They require no ripening before they are consumed and may be regarded simply as a special form of sour milk. Such cheeses cannot be kept for very long periods because molds and yeasts begin to develop which soon spoil the flavor and render the cheese unfit for use.

Rennet Curd Cheeses. — A great variety of cheeses is produced by the action of rennet upon milk. A dozen or more are commonly sold on the market, and several hundred in addition have been described. Differences among these cheeses are due to several factors: the differences in the source of the milk, *i.e.*, whether from the cow, goat, sheep, etc.; the amount of moisture retained with the curd; the amount of salt, and the nature of certain condiments added; the size of the cheese cake; the temperature and conditions of ripening; the presence of certain specific organisms, particularly molds and bacteria, in some cases intentionally added and in others normally present under the conditions of manufacture.

The cheeses produced by the action of rennet may be divided into two classes, the *hard* and the *soft*. The primary difference between these classes is the amount of whey or moisture left in the curd.

The commonest type of hard cheese is the so-called *Cheddar cheese*. The milk used in the production of this cheese must be of good quality. Particular care must be used that gas-forming

bacteria of the *Bact. coli* and *Bact. aërogenes* types are not present. A certain amount of acid is usually allowed to develop before the rennet is added, commonly about 0.2 per cent. Rennet is added to the milk, which quickly curdles. The curd is cut into small pieces by means of knives; it then shrinks and expels the whey. The acid-producing bacteria continue to develop. The acid unites in part with the casein; the latter changes in consistency somewhat, and unites to form a coherent mass. The whey is largely removed by pressing the curd into cakes of different sizes. The cheese is then placed in a curing room and allowed to remain until the ripening process is complete.

This ripening process is probably due to a combination of factors. Normal milk contains a proteolytic enzyme, *galactase*. The rennet added to the milk contains more or less *pepsin*, likewise a proteolytic ferment. Certain *bacteria* may also secrete proteolytic enzymes, though their importance has been brought into question. The first agency of importance is probably the bacteria producing lactic acid. These continue to multiply until the milk sugar has all been used up. The lactic acid formed combines with the casein. The galactase and pepsin continue their action. The pepsin is active only in the presence of acids, hence the development of these acids creates favorable conditions for its activity. It is not entirely certain whether bacterial enzymes are responsible in this cheese for any of the proteolysis, but such is not impossible although no species have thus far been found that can satisfactorily duplicate in pure culture the changes which take place in the cheese itself. The proteolysis or digestion of the casein results in rendering the curd relatively soluble. At the same time, flavoring substances, the nature of which is not thoroughly understood, are developed. In some cases, these are in part ammonia and related compounds, and in part the ethyl esters of the fatty acids, particularly the volatile acids. It is possible that the autolytic enzymes liberated by the death of the lactic acid bacteria may also assist in the development of flavor.

When bacteria of the *Bact. coli* type are present in considerable numbers, gas bubbles form in the curd, and foul-smelling compounds develop. These organisms are generally present in

small numbers in milk, and cheese usually contains some gas bubbles, but these are not numerous. Yeasts which can ferment lactose with gas production have also been described from cheese.

Camembert cheese may be taken as a type of soft cheese. The curd is produced as has been described for Cheddar cheese, but is allowed to drain without being cut up or heated, thus retaining a much larger proportion of the whey. The cheese is molded into small cakes and placed in a curing room, the temperature

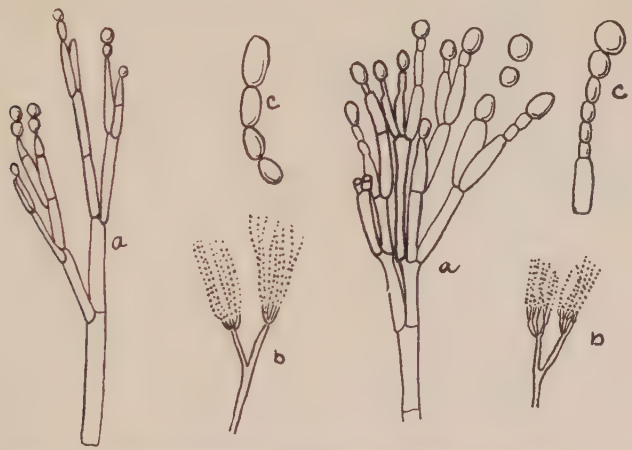


FIG. 143. — 1, *Penicillium camembertii* from Camembert cheese. 2, *P. roquefortii* from Roquefort cheese. *a*, characteristic branching of the conidiophores. *b*, same on smaller scale. *c*, conidia. (Adapted from Thom.)

of which is usually not very low. Molds soon cover the surface of the cheese, the *Oöspora* (*Oidium*) *lactis* developing first, and later varieties of *Penicillium*, particularly *Penicillium camembertii*. These molds, as has been noted previously, are capable of utilizing organic acids as food. The acidity of the cheese is thus gradually reduced, and at the same time the molds secrete proteolytic enzymes which gradually diffuse toward the interior of the cheese. The mycelium, however, ordinarily does not penetrate the cheese to any considerable distance. When the proteolysis occasioned by this mold development has penetrated to the center of the cheese, it is ready for use. It will usually not keep long because the reduction

in acidity allows the development of putrefactive bacteria. It seems probable that the molds in this case are much more important than the enzymes ordinarily present in the cheese. It is believed that the proteolytic changes in the texture of this cheese ripening are due to the Penicillium, while the characteristic flavor has been ascribed to the Oöspora (Oidium).

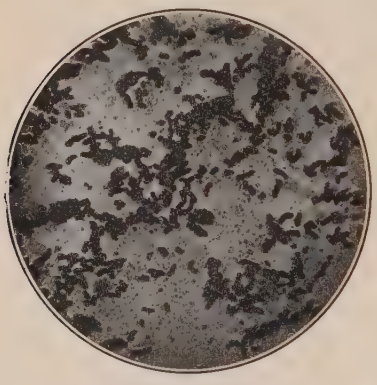


Fig. 144. — Bacteria from the surface of Limburger cheese. (× 1000.)

Roquefort cheese as originally prepared was made from sheep's milk, but a similar cheese is made from the milk of the cow. The cut surface of this cheese shows a characteristic greenish mottling due to the presence of a specific mold, *Penicillium roquefortii*. The cheese is inoculated with this organism by allowing bread to mold and mixing the moldy bread crumbs with the curd. The Penicillium is an aërobic organism, so to facilitate its growth within the cheese, holes are punched by means of stiff wires.

Limburger Cheese. — The distinct and characteristic flavor of certain cheeses (among them Limburger cheese) is due to the continuance of the ripening process until the bacterial digestion and decomposition have proceeded further than in most other cheese. The slimy layer on the surface of such cheeses is composed largely of masses of putrefactive bacteria.

CHAPTER XXXI

CHEMICAL SYNTHESES BROUGHT ABOUT BY MICROÖRGANISMS

Cell Wall and Capsules. — The cell walls of most bacteria and molds are nitrogenous, but in some cases they give the characteristic reactions of cellulose. The nitrogenous wall is frequently termed *chitinous*. When much thickened, the outer swollen capsule may reach a diameter several times that of the cell itself. The principal point of economic importance with reference to this synthesis is that this capsular material may produce certain changes in the physical structure or consistency of the medium. These have already been discussed in the preceding chapter. Chemically these materials may be divided into two groups, the mucins and the carbohydrate polymers or gums.

The mucins are the slimes produced by certain organisms when growing in a protein- or peptone-rich medium such as milk or beef broth. They may be identified by precipitation with alcohol, followed by re-solution in water, repeating the process several times. The mucins give most of the characteristic protein reactions. When heated with acids they acquire the property of reducing Fehling's solution, showing them to contain a carbohydrate radical. They evidently are complex nitrogenous compounds breaking up readily into proteins and carbohydrates. Mucins have been described as one of the causes of slimy milk, but it is probable that in most cases this is due to the development of gums of the type to be next described.

The gums or gumlike carbohydrates synthesized by microorganisms are of many types, depending perhaps in part on the carbohydrate present in the medium. Mannans and dextrans, polymeric anhydrides of mannose and dextrose respectively, are among the commonest. Galactans and levulans also have been

noted. These gumlike substances either swell considerably in water or dissolve in it more or less completely. They are precipitated by alcohol. When heated with acid they break down into simple sugars which give the Fehling reaction. Chemically they are closely related to the vegetable gums; in fact, as has been noted, there is some reason for believing some of these latter to be formed by bacterial and mold action. These substances are frequently met with wherever bacteria, yeasts, and molds have grown. They are particularly common in saccharine solutions such as wine, beerwort, milk, and even in the syrups used in the manufacture of sugar. They are therefore of considerable significance in certain industries, as they sometimes are the cause of serious loss.

Synthesis of Nitrogenous Compounds. — Microörganisms may synthesize nitrogen into complex compounds of several types, the most important being the protoplasm of the organism, enzymes, mucins, and probably toxins and endotoxins. The protoplasm and enzymes have already been discussed.

Mucins are compounds produced as a capsular material by a few species of bacteria. They resemble the bacterial gums in origin, but are nitrogenous. The cause of slimy milk, and other slimy liquids with high nitrogen content, is frequently the mucins produced by certain species of bacteria.

Toxins are to be sharply differentiated from the poisonous ptomaïnes discussed above. They are the products of synthetic activity; their chemical composition is unknown; they can be tested only by biological means (animal injection); they are easily destroyed by heat, and when injected in repeated nonlethal doses into a suitable animal should stimulate the production of an antitoxin which is capable of neutralizing the toxin. The toxins will be considered at greater length in Chapter XXXIII on Immunity.

Endotoxins are also products of synthetic action. Like toxins they are poisonous, but unlike them are frequently retained within the cell of the microörganism. Antitoxins are not formed for endotoxins when these are injected into the bodies of animals.

Pigments. — Many yeasts, molds, and bacteria are said to be *chromogenic*, that is, they produce *pigments*, or coloring matter.

A few bacteria contain within their bodies a pigment already discussed, *bacteriopurpurin*, a red-purple coloring matter that enables the organism to assimilate carbon dioxide by the aid of the absorbed sunlight. In all others the pigment is to be regarded as a secondary waste product. In most cases, the pigment produced is insoluble in water and remains within the cell or cell wall. The mycelium and spores of one whole family of molds, the Dematiaceæ, are brown or fuscous, the spores of many other molds are of bright colors such as green, yellow, orange, and red. Bacteria producing pigment of this type are abundant, particularly the red and yellow forms, though in addition species are known that duplicate practically every color of the spectrum. Some of these are of economic importance, such as *Serratia marcescens* which produces red spots and blotches on carbohydrate foods, such as bread, and is responsible for the so-called "bloody bread." Blue milk, red milk, and red spots on cheese are also due to bacteria. Pink, brown, and black yeasts are not uncommon.

Pigments are usually classified by their solubility in various agents, such as water, ether, alcohol, and chloroform. Chemically they belong to very different groups. Some are *lipochromes* or fatty pigments resembling those of the petals of some flowers; others are quite insoluble in the common fat solvents.

Most organisms produce pigments only in the presence of free oxygen, although there are some chromogenic anaërobes. The property of pigment production may be lost by an organism, and it may not be recovered even by cultivation under conditions most favorable for its development.

SECTION V

MICROÖRGANISMS AND HEALTH

CHAPTER XXXII

GENERAL DISCUSSION OF DISEASE

Several theories of disease have been held during ancient, medieval, and modern times. The theory which is most generally accepted among savages and semicivilized tribes, and which was once universally accepted, may be termed the *demonic*. Evil spirits were believed to invade the body, or in some cases disease was attributed to the direct displeasure of the gods. Exorcisms, spells, amulets, charms, etc., were commonly used to avert disease.

Hippocrates, generally termed the father of medicine, developed the so-called *humoral* theory of disease which held sway until comparatively recent times. According to his theory the body contained four humors: blood, phlegm, yellow bile, and black bile. When these were mixed in the proper proportions, the body was considered to be in a state of health. When one gained the ascendancy and there was an improper mixture, the body was believed to be diseased. Many of the methods of treatment used by physicians in olden times were based upon this theory, such as that of bloodletting. This theory began to break down in the sixteenth century, and during the next two centuries many others were advanced to take its place. Of these various theories, there is only one which persists at the present time, that of Hahnemann, the founder of the homeopathic school of medicine.

One theory which gained considerable favor during the past century and which has exerted and still exerts a marked influence upon our methods of sanitation, is the *pythogenic*. It was developed about the middle of the nineteenth century by Murchison, an Englishman. According to this theory disease always originates in dirt or filth of some kind. Disease-producing organisms were supposed to originate *de novo* in such material, or at least they found

favorable conditions for their multiplication. Heaps of decaying organic matter were held to be centers of distribution for harmful microörganisms. Considerable emphasis was likewise laid upon the ability of organisms to be carried through the air by sewer gas, etc. Later, this theory was somewhat modified and was finally regarded as an explanation of the spread of certain of the fevers. Murchison's definition of typhoid fever is "an endemic disease generated and propagated by certain forms of decomposing matter. It may be generated independently of a previous case by fermentation of fecal and perhaps other organic matter. It may be communicated by the sick to persons in health, but even then, the poison is not like that of smallpox, given off from the body in virulent form, but is developed by the decomposition of the excreta after their discharge. Consequently an outbreak of typhoid implies poisoning of air, water, and other ingesta with the decomposing excrement." Emphasis should be laid upon the fact that he believed diseases of this type could originate without any preëxisting disease of the same type to serve as a source of the virus.

The theory of disease which is now commonly held and which is the only one which rests upon a firm foundation of fact is the *germ* theory. The researches of Louis Pasteur upon the causes of various types of fermentation, such as the production of alcohol and carbon dioxide by yeasts from certain sugars, and the transformation of the lactose in milk to lactic acid by bacteria, suggested that specific organisms might in a similar manner produce disease in man and animals. It was shown early in the nineteenth century that the disease known as favus, a kind of ringworm of the scalp, was due to a specific fungus, and one type of silkworm disease was later shown to be caused by a fungous parasite. Within the last sixty years the specific causes of a great many of the diseases of man and animals have been determined, although there are still some which thus far defy the efforts of investigators. It must be recognized that the germ theory of disease does not insist that all diseases are caused by microörganisms. There are many diseases in which there is probably no cause other than a constitutional defect of some kind wholly apart from microörganisms of any kind. Diabetes, certain kinds of indigestion, rickets, and dietary

deficiency diseases in general are certainly not caused in any direct way by microörganisms.

The channel through which a disease-producing organism gains entrance into the body is termed its *infection atrium*. Organisms may enter through the alimentary tract, the respiratory tract, through the skin by means of wounds, or through the skin glands. Some of the most important diseases are transmitted by ingestion, the carrier of the infection ordinarily being water or food. Diseases of the intestinal tract, such as typhoid, dysentery, and Asiatic cholera, are universally acquired by this means, and some others, such as tuberculosis, may be contracted in this manner. Air usually contains bacteria, yeasts, and molds. Only under exceptional conditions are these forms pathogenic. Certain organisms, however, when inspired with the air may gain foothold in the mucous membranes of the respiratory tract and produce disease. This is believed to be one of the common methods of contracting tuberculosis. Many of the diseases at one time supposed to be transmitted by the inhalation of the organism have since been shown to be acquired by ingestion. A few disease organisms may attack the healthy uninjured skin. Among them are certain of the molds producing ringworm and similar diseases. A few disease organisms, too, are transmitted by direct inoculation into the body. Tetanus or lockjaw is acquired by the introduction of the organism into a wound and probably in no other way. Certain diseases are transmitted only by insects. Malaria and yellow fever, for example, are never transmitted otherwise than by direct inoculation into the body by mosquitoes. Certain blood-sucking flies transmit other diseases, such as the sleeping sickness of man and related diseases of animals in Africa and in tropical countries. Flies may also serve as carriers of disease-producing organisms from the filth in which they breed to food used for human consumption.

Classification of Diseases. — *Infectious Diseases.* — Diseases may be divided into two groups, infectious and non-infectious. An infectious disease is one that is caused by a microörganism. A non-infectious disease is one that is not so caused. Popularly the term infectious is often used to indicate the method of transmission of a disease. Literally, it has nothing directly to do with

such transmission. It is simply necessary that an organism be the specific cause of a disease in order that that disease shall be termed infectious. A considerable proportion of the diseases known are infectious. Such, for example, are erysipelas, diphtheria, typhoid fever, smallpox, Asiatic cholera, bubonic plague, whooping cough, dysentery, tuberculosis, pneumonia, anthrax, tetanus, etc. Among the common non-infectious diseases may be enumerated diabetes, Bright's disease, some indigestions, heart troubles of some types, certain nerve affections, etc. An individual harboring a disease-producing organism is said to be *infected* by the organism. Any article of clothing or inorganic material of any kind which may serve to harbor the organism and aid in the transmission of the disease (that is, any *fomes*) is said to be *infective*. For example, a public drinking cup used by a tubercular individual may be termed infective, while the individual himself is said to be infected.

Contagious Diseases. — Infectious diseases may be subdivided into those that are contagious and those that are non-contagious. A contagious disease is one that is readily transmitted by direct or indirect contact with the diseased individual. A non-contagious disease is one which is not readily transmitted in this manner. All gradations may be found between these two classes of diseases, for we speak of some diseases as being highly contagious, others as mildly contagious. Relatively few of the infectious diseases are strictly non-contagious. They are for the most part those diseases which require direct inoculation for their transference. Among them may be listed tetanus or lockjaw, malaria, and yellow fever.

Disease Types produced by Microörganisms. — The changes brought about by the growth of disease-producing organisms in the body are quite varied, differing quite as much as the morphologic and cultural characters of the organisms when grown outside of the body. Certain classifications of types of diseases, however, prove useful in discussion.

Diseases are sometimes grouped as *specific* and *non-specific* infections. A specific infection is one characterized in every case by a definite sequence of changes and reactions on the part of the body and produced by a definite species of microörganisms. Such

diseases as typhoid fever, diphtheria, and tuberculosis are included here. A non-specific infection, on the other hand, is one that may be produced by one of several species of organisms, and which does not run a clinical course whereby the organism causing it can be very definitely identified. Such diseases would include common colds, pus production in wounds, etc.

A disease caused by a single species of organism working alone in the body may be termed a *primary* infection. Sometimes two or more organisms may be found together producing disease. Such would be termed a *mixed infection*. In some cases, infection with one species of organism may so weaken the resistance of the body that it readily becomes a prey to some other disease. For example, pneumonia not infrequently accompanies or follows typhoid fever and measles. The organism causing pneumonia is totally distinct from those causing the other diseases, but finds opportunity for development when the body has become weakened. Such a disease is termed a *secondary* infection.

Diseases are also classified by the bacteriologist and pathologist into several groups depending upon the location of the organism in the body, whether or not it produces poisons or toxins, and upon its distribution through the tissues. A *toxemia* is a disease in which the organism remains localized and produces a poison or toxin which enters the blood stream and causes damage to tissues in other parts of the body. For example, the diphtheria organisms rarely leave the throat but produce a toxin which enters the blood stream and causes damage to the heart muscles, the liver, and other tissues. In certain diseases or infections the organisms remain localized but do not form an appreciable amount of toxin. Such are wound infections and pus production in general. Diseases in which the blood stream is generally invaded are termed *bacteremias*. Such general invasion may occur in typhoid fever. The term *septicemia* is frequently used synonymously with bacteremia, although some authors confine it to those diseases caused primarily by pus-producing organisms in which the bacteria multiply in the blood stream. A *sapremia* is a disease produced by the absorption of poisonous putrefactive products from dead or decayiug tissue. Such might result from gangrene, or from a retained placenta.

Those diseases in which there is an eruption of the skin, as smallpox, chickenpox, etc., are termed *exanthemata*.

Uses of Animals and Animal Inoculation in the Bacteriological Laboratory. — It is sometimes necessary to make use of animals in experimental work in bacteriology. This is not a matter of choice with the bacteriologist or pathologist, for there appears to be no other way open for the determination of certain facts. The most important reasons for the use of animals are enumerated below. *First*, inoculation of animals is sometimes necessary to recognize or diagnose certain diseases. For example, one of the most reliable means of determining whether or not a dog has rabies, that is, whether or not it is mad, is by the injection of a small portion of the brain tissue into a rabbit. The outcome of such an injection may determine the treatment of individuals that have been bitten by such a dog. *Second*, it is necessary in the initial study of a disease to inject organisms suspected of causing the disease into animals to prove or disprove such relationship. Had it not been for animal inoculation, it would have been impossible to have determined the causes of such diseases as diphtheria and tuberculosis. *Third*, it has already been noted under the discussion of the methods of securing pure cultures of bacteria that it is sometimes necessary to inoculate organisms into the animal body in order to eliminate all the forms which are not capable of producing disease. For example, in the isolation of the bacillus of tuberculosis from milk, the simplest method is to inject some of the sediment secured from the milk into a suitable animal, such as the guinea pig, and allow the disease to develop. Later when the animal is killed, the organism may be found in pure cultures in the nodules characteristic of the disease. *Fourth*, animals are used for the manufacture of certain products designed for the treatment and prevention of disease, such as antitoxins and other antisera. These will be discussed at greater length in the next chapter. *Fifth*, animals are essential in testing certain biological products. The only method that has been evolved for determining the strength of toxins and antitoxins is by animal injection. The chemist has never been able to identify and isolate these materials. The animal is used by the bacteriologist in the laboratory in much the same manner as

the chemist makes use of an indicator to determine the acidity or alkalinity of a solution.

The animals most commonly utilized in the bacteriological laboratory are the guinea pig and the rabbit. Mice and rats are used for some purposes. In the investigation of a few diseases, particularly those infecting birds and fowls, the pigeon and domestic fowl are used. Monkeys have been utilized for the study of some human diseases which cannot be communicated to any of the other lower animals. Calves are used for the preparation of smallpox vaccines. The horse is the common source of antitoxins.

Inoculation of animals is usually accomplished by the use of the hypodermic syringe and needle. The material may be injected under the skin (*subcutaneously*), into the veins (*intravenously*), into the abdominal or peritoneal cavity (*intraperitoneally*). Occasally organisms may be injected into the thoracic cavity or into the brain (*sub-durally*). Animals may be infected also by causing them to *ingest* or *inhale* the microörganisms being studied.

Koch's Postulates. — Dr. Robert Koch, the eminent German bacteriologist, formulated certain rules that should be followed whenever possible for determining the relationship between a specific organism and a particular disease. It is not a simple matter in all cases to demonstrate conclusively that a particular organism causes a certain disease. Koch's postulates may be enumerated as follows: *First*, the organism suspected of causing the disease must be found in all cases of the disease. *Second*, this organism must be isolated from the diseased individual and cultivated in pure culture. *Third*, pure cultures of the organism when injected into a suitable animal must reproduce the disease. *Fourth*, the specific organism in question must again be isolated from the diseased individual. It is not in all cases possible to carry out all these postulates. For example, there are diseases of man which are not communicable to lower animals, hence the third postulate cannot be satisfied. Then again there are organisms producing disease which are ultramicroscopic and still others which have so far never been cultivated upon artificial media. Several other lines of evidence have been utilized for many of these diseases, but in some cases the relationship of organism to disease is still an unsettled question.

CHAPTER XXXIII

RESISTANCE OF THE BODY TO DISEASE

THE resistance to infection which is offered by the body is termed *immunity*. Certain facts of immunity are familiar to all. It is well understood, for example, that an attack of a disease such as smallpox renders the individual relatively immune or quite resistant to subsequent exposure to that disease. Many of the diseases attacking the lower animals are not communicable to man. The converse of immunity, that is, the lack of resistance to disease, is termed *susceptibility*.

Natural Body Barriers to Infection. — The *skin* is a comparatively efficient barrier to the ingress of microörganisms. It is continually being renewed from below and scaling off at the surface. Microörganisms frequently penetrate to some depth in the skin, but rarely gain entrance to the living tissues below. Very few pathogenic organisms can penetrate through the unbroken skin. Occasionally the organisms pass through the glands or through the hair follicles and produce localized infections such as boils and carbuncles. Just under the skin there are layers of *fascia* or connective tissue which also afford an excellent mechanical barrier to the penetration of microörganisms. These are, moreover, infiltrated with serum, and this serum, as will be shown later, may aid in the destruction of organisms.

The mucous membranes lining the body cavities which communicate with the surface are usually not easily penetrated by organisms. They derive their name from their secretion of mucus, thrown off by certain cells termed goblet cells. This mucus catches and retains particles of dust and organisms of all kinds that come in contact with it. This is particularly marked in the respiratory tract, where the mucus is secreted in considerable quantities by the membrane lining the tracheal and bronchial tubes. Organisms from

the air are caught on this moist surface and the mucus is continually being swept up toward the mouth by minute cilia projecting from certain of the cells, constituting the so-called ciliated epithelium. Microörganisms also find mechanical barriers to their entrance with the inspired air in the *hairs* which are just inside the nose, and serve as filters.

In the mouth there are great numbers of microörganisms normally to be found. *The acid secretions* of the gastric glands are sufficiently antiseptic so that few bacteria can multiply in them, and many bacteria, though by no means all, in food and water ingested are destroyed during passage through the stomach. When the contents of the stomach pass into the intestine, the acid is neutralized by the alkaline pancreatic and other intestinal juices. The reaction becomes therefore more favorable for bacterial growth, but most organisms are inhibited from developing by the fact that the *bile* is antiseptic and will prevent the growth of most species. This is not true of all species, however, as some of the bacteria (as *Bacterium coli*) normally present in the intestines and some of those which produce intestinal disease and gain entrance to the body through the alimentary tract, as *Bacterium typhosum* of typhoid fever and *Bact. dysenteriæ* of dysentery, are not inhibited in their development by this material. In fact, it will be found in later discussions that a mixture of bile and sugar is used as a culture medium for some of these intestinal bacteria. In addition to these natural body barriers to infection, various other factors tending to prevent the entrance of disease-producing bacteria into the body will be discussed later.

Factors Predisposing to Disease. — It is a matter of common knowledge that every individual differs markedly at various times in his ability to resist bacterial infection. *Age* is frequently one of the most important predisposing factors to disease. There are certain diseases which are very easily acquired by children and somewhat more rarely by adults. Such are scarlet fever, whooping cough, and measles. On the other hand, there are certain diseases which are much more common among adults, for example, cancer and diabetes. *Hunger* and *thirst* both decrease body resistance. Exposure to *heat* or chilling of the body surfaces by *cold* may also be

predisposing factors to disease. It is found, for example, that if any ordinary barnyard fowl is kept in cold water for some time, it loses its natural immunity to such diseases as tetanus. Excessive *fatigue* also predisposes to disease. This has been many times experimentally demonstrated in the laboratory. It is found, for example, that if a white rat which is normally immune to the disease anthrax is worked in a treadmill until completely exhausted, then injected with this organism, it will contract the disease and succumb to it.

The ability of a particular organism to produce disease depends upon several factors. *First* of these is the *virulence* of the organism, that is, its relative pathogenicity (disease-producing or attacking power). It is found in some cases that growing microörganisms under unfavorable conditions or subjecting them to the action of heat or chemicals decreases very considerably their ability to produce disease. It is well known, for example, that in some epidemics of a particular disease, such as diphtheria, the organisms isolated from some infected throats are less virulent than from others. That is, they have less disease-producing power. *Second*, the ability to produce disease may depend upon the *number* of organisms introduced into the body. The exact number necessary to produce the disease will depend upon the kind of organism. In some cases, as in anthrax, the introduction of a single organism into the blood stream of the animal may cause a fatal case of the disease. In other cases, there are no symptoms unless a considerable number of the organisms are introduced. *Third*, the ability of an organism to produce disease is sometimes dependent upon the infection atrium or channel through which it is introduced into the body. The tetanus bacillus, for example, may be swallowed with impunity; it can develop and produce the characteristic symptoms and lesions of the disease only when introduced into a wound. *Fourth*, the ability to produce disease is also dependent upon the relative *resistance* of the particular individual infected. This resistance or immunity may be subject to considerable fluctuations in the same individual.

Inheritance of Disease. — It has never been satisfactorily demonstrated that any infectious disease is ever inherited in the

strict meaning of the term. This does not mean that an individual may not be diseased at birth: as a matter of fact, this not infrequently occurs, but disease is never inherited in the same manner as are various body characteristics. It is not transmitted through the germ plasm. It is true that predisposition to disease may be inherited. We sometimes hear the expression "tuberculosis (or consumption) runs in the family." This means simply that the predisposition to the disease is inherited, for very rarely indeed is an individual tuberculous at birth. Then the intimate contact of members of a family with each other renders the spread of tuberculosis from one individual to another comparatively easy. It is difficult to tell in all cases just how much the prevalence of a disease in a certain group or family may be due to actual predisposition to the disease on the one hand, or to the unusual opportunities for infection on the other.

If disease is defined as any abnormality of the body or the functioning of its parts, then certain non-infectious diseases may be termed inherited. Such, for example, may be color-blindness.

One example of pseudo-inheritance of an infectious disease is white diarrhea of chicks. In this disease certain hens are "carriers," that is, they have the causal organism permanently present in the ovary. In consequence the eggs laid are infected, and the chick is infected before hatching. This would seem at first to be an example of infectious disease inheritance. It is not such, strictly speaking. The true hereditary mechanism of the cell is not changed.

Types of Immunity. — Immunity may be divided into two distinct types — *natural* and *acquired*. The former may be subdivided into *racial* or specific immunity and *individual* immunity; the latter into *active* immunity and *passive* immunity.

Natural Immunity. — Natural immunity is immunity that an individual has from birth; that is, it is not acquired after birth. For example, we know that members of certain groups of animals are wholly immune to diseases of other groups. Fowls are normally immune to tetanus or lockjaw, the domestic animals never have typhoid fever, and man does not contract horse distemper or hog cholera. Even within a certain group it may be found that

some races are more resistant than others. It is well known, for example, that the negro is much more resistant to malaria than the white. On the contrary, the white is probably more resistant to tuberculosis than the negro. In any epidemic of disease in man, a certain number of individuals will always be found to escape, because for some reason they are naturally immune to the disease.

Acquired Immunity.—Acquired immunity may be either *active* or *passive.* An *active acquired immunity* is one which is brought about by the development in the body of the individual of certain substances which render him immune. A *passive acquired immunity* is one which is conferred upon an individual by the injection of immune substances produced by another animal or another individual. One who is passively immunized to a disease takes no part in the production or development of this immunity. For example, a person who has diphtheria is after recovery relatively immune to the disease because the body itself has produced immune substances. Such an individual is said to have an active acquired immunity. On the other hand, an individual may be rendered immune for a shorter or longer period by the injection into the body of antitoxins or other immune substances developed in the blood of certain animals. This is termed passive immunity, for the body of the individual thus immunized takes no part in the development of the immunity.

Active Acquired Immunity. — Active immunity may be acquired in any one of several ways, the method depending very largely upon the type of disease. There are certain diseases which confer upon an individual who has them an active immunity to a recurrence. This is true in such diseases as smallpox, measles, whooping cough, etc. Immunity may also be acquired by *vaccination.* This term is popularly used most commonly in connection with the disease smallpox, but vaccines have been developed which are useful against other diseases of man and animals. By vaccination is meant the injection or inoculation of dead or living organisms into the body of the individual. If living organisms are used, they are attenuated, or rendered inactive or unable to produce a severe type of the disease. In other words, vaccination in such cases is the production in an individual of an infection that will run a benign

course. Vaccines are used commonly for the prevention of diseases, much more rarely for their cure. Vaccination against typhoid fever, for example, is accomplished by growing typhoid bacilli, suspending them in solution, killing them by heat, then injecting them subcutaneously into the individual to be immunized. The body reacts in much the same manner as though the individual had a mild case of typhoid fever and a considerable degree of immunity is developed. Active immunity may also be acquired by the injection of the products of some kinds of microörganisms. The toxin or poisonous substance produced by the diphtheria bacillus, for example, if injected several times in suitable doses, will cause an individual to become immune to the disease itself.

In some cases active immunity is *localized*. In certain infections the area of body tissue involved may be circumscribed, that is, rather definitely limited, and it is found that tissues that have been thus involved have developed a local resistance to a recurrence of the infection, but that such resistance has not been developed by all parts of the body.

Passive Acquired Immunity. — Immunity may be acquired passively only by the injecting of immune substances directly into the individual. These immune substances are in general the products of the active immunization of an animal. For example (as will be later shown), the antitoxin for diphtheria is produced by actively immunizing a horse against the disease, then using the blood serum of the animal for the injection of the human. Passive immunization is of considerable assistance in the cure and prevention of a few diseases, but unfortunately is not applicable to most diseases.

Theories of Immunity. — The fact that individuals become immune to certain diseases has been known from the earliest times. It is only within modern times, however, that specific theories have been promulgated to explain this immunity. Four of these theories require brief mention, two of them for their historical importance, and two because they are the ones now held to explain the various phenomena.

Exhaustion Theory of Immunity. — When bacteria, yeasts, and molds were first cultivated in media of various kinds in the labora-

tory, it was found that growth would not continue indefinitely in a given culture medium, but would cease in the course of a few days or weeks. It was believed that this was due to an exhaustion of the food material in the medium. A similar explanation was suggested to account for immunity in man and animals. It was believed that certain substances essential to the development of the particular disease-producing organism were found in the body and that the organism could grow only as long as these were present. When these substances were exhausted, the individual was thereafter immune because the organism could no longer develop in the body. It was soon discovered, however, that even in the laboratory plenty of food material could be demonstrated in tubes in which all growth had ceased, and it was further noted that the blood serum from an individual immune to certain diseases could be utilized as a culture medium for organisms of the same type, proving conclusively that immunity is not due to the complete removal of any particular food substance.

Noxious Retention Theory. — It was soon ascertained that microörganisms cease growing in a culture medium not because of the exhaustion of food material, but because of the production by every living cell of substances more or less inimical to its own growth. It is generally true that the waste products of any cell are injurious to it, and where such tend to accumulate, as in the culture medium of a test tube or flask, they soon stop all growth completely. A similar explanation was applied to immunity. It was urged that particular pathogenic bacteria when growing in the body produce substances harmful to themselves; these substances accumulate and finally stop growth. This theory, however, was soon replaced by the two next to be considered.

Metchnikoff's Theory of Phagocytosis. — Metchnikoff observed that some of the cells of the body, particularly certain types of white blood corpuscles or *leucocytes*, have the ability to swallow or ingest bacteria and foreign particles of many kinds. These cells he termed *phagocytes*, and the phenomenon of ingestion of the bacteria by the cells, *phagocytosis*. He believed immunity to be due to a process of education or training whereby the phagocytes of the body acquired the capacity of ingesting and destroying patho-

genic bacteria of a certain type whenever they gained entrance to the body. This theory in a somewhat more elaborate form and modified in some respects by the theory next to be described, is generally held at the present time. The white blood corpuscles are rightly called the "policemen of the blood."

Ehrlich's Humoral Theory. — Ehrlich came to the conclusion that immunity is due to the development in the body fluids (*i.e.*, the body *humors*) of certain specific substances. These substances, so significant in the production of immunity, he called *antibodies*. He found that the injection of many kinds of materials into the body would cause the appearance in the blood or other body fluids of substances that would react with them, in some cases neutralize them; in other cases, destroy them; in still other cases, precipitate or dissolve them. Any material injected into the body for the purpose of producing these substances is termed an *antigen*, while any substance formed as a result of the injection into the body of the antigen and reacting with it is termed an *antibody*. For example, the toxin of the diphtheria bacillus when injected into the body in small quantities as an antigen causes the tissues to produce an antibody called *antitoxin* which is capable of neutralizing it. The antibodies produced are of several types: those capable of neutralizing poisons or toxins are called *antitoxins;* those that can cause *clumping* (*agglutination*) or *precipitation* are called *agglutinins* and *precipitins;* those which can dissolve or destroy cells are termed *cytolysins* or *cytotoxins;* and those which stimulate *phagocytosis* are called *opsonins*. Some have also been described which are chiefly concerned in rendering the body sensitive or highly susceptible. These will be considered in order.

Antitoxins and Related Antibodies

Toxins. — The word *toxin* is used in several different senses. In some cases it is used essentially as a synonym of poison. As employed by students of immunity it indicates a particular type of poisonous substance. It is used here in this restricted sense. Toxins all have certain characteristics in common. The characteristics of a true toxin may be formulated as follows:

1. Toxins are poisonous organic substances secreted by living cells of plants or animals. This definition excludes, of course, all poisons of inorganic origin, such as arsenic.

2. Toxins when injected in non-fatal doses into suitable animals cause the animal to produce in its blood or tissues substances called *antitoxins* which will neutralize this poison. In consequence the animal develops a very high degree of immunity toward the toxin. This excludes from consideration many poisonous substances produced by plants and animals which do not have this characteristic. For example, the poisons opium and strychnine produced by plants do not cause the body to produce substances which will neutralize or destroy them.

3. Most toxins are *thermolabile;* that is, they are easily destroyed by heat. Practically all of them are rendered inactive by brief exposure to the temperature of boiling water, and some of them are destroyed at temperatures far below this. They are also usually destroyed by exposure to light, to oxygen, and to various chemicals.

4. The chemical composition of toxins has not been accurately determined. It has thus far proved impossible for the chemist even to determine the presence of toxins in solution by the use of chemical means alone. The only method so far devised for their detection and study is that of *animal inoculation*. In short, toxins must be investigated principally by biological or bio-chemical methods.

5. The introduction of a toxin into the body generally causes no body reaction until some time has elapsed. In other words, toxins are said to show a definite *incubation period*. This is because the toxin after gaining entrance to the body must combine chemically with certain cells for which it has an affinity before damage can be noted.

Toxins are produced by many plants and animals. The venom of the rattlesnake and cobra, the poison of the scorpion and the tarantula, the poison produced by certain fishes and insects, are all animal toxins or *zoötoxins*. Toxins produced by plants (*phytotoxins*) are also numerous. The juices of the castor oil bean and the bark of the locust tree contain very virulent and powerful toxins known as *ricin* and *robin* respectively. Most important for con-

sideration here is the fact that a few bacteria likewise produce toxins. The principal pathogenic organisms that produce these toxins are the diphtheria bacillus (*Corynebacterium diphtheriæ*), the tetanus bacillus (*Clostridium tetani*), some of the bacteria of meat poisoning or botulism (*Clostridium botulinum*), probably certain forms of dysentery bacilli (*Bacterium dysenteriæ*) and the streptococcus of scarlet fever (*Streptococcus scarlatinæ*).

Not all poisons produced by bacteria are true toxins; some do not answer in their characteristics to those enumerated above. In some cases they are *ptomaïnes* resulting from putrefaction or decay; in others they are poisonous products whose nature has not been established definitely, termed *endotoxins*. These endotoxins are usually closely bound up with the protoplasm of the cell and can be released only by death and autolysis. These endotoxins differ chiefly from the toxins in that when injected into the animal body, they do not cause the production of antitoxins. Endotoxins will be found to be of considerable importance in connection with food poisoning, particularly that produced by *Bact. enteritidis*.

Preparation of the Diphtheria Toxin. — Our principal interest in toxins of disease-producing organisms lies in the fact that they must be prepared as a preliminary to the production of antitoxins. The most commonly used of the antitoxins and the one whose preparation may be used to illustrate the preparation of all others is that specific for diphtheria. The diphtheria bacillus is grown in large, flat-bottomed flasks containing a relatively shallow layer of a specially prepared nutrient broth. Much study has been devoted to the composition of this broth for the purpose of securing a medium which will allow the maximum production of toxin. The organism is inoculated upon the medium, where it grows as a scum over the surface. It is essential that plenty of oxygen be furnished to secure a maximum production of toxin, hence the relatively large area of the medium exposed to the air. This is then incubated at blood heat until the film of diphtheria bacilli has spread completely over the surface. It is then removed and a little carbolic acid added to destroy the bacteria present. It is filtered through a porcelain filter to remove the bodies of the bacteria. The toxin

remains in solution. It is necessary then to determine the concentration of the toxin, inasmuch as no two flasks will be found to contain exactly equal amounts. It has already been noted that toxins can be studied only by biological methods, hence the only method of determining the strength of toxin is by injection into animals. Guinea pigs weighing two hundred and fifty grams are commonly used. A number of these animals are injected with varying amounts of the toxin and the smallest fatal dose accurately determined. This dose is known as the *minimum lethal dose* (abbreviated as M.L.D.). Knowing this, the manufacturer of antitoxin can ascertain certainly how much may be injected with safety into an animal such as the horse, which he intends to use for antitoxin production.

Preparation of Diphtheria Antitoxin. — It is customary to use the horse in preparation of diphtheria antitoxin. For this purpose normal healthy horses are secured and every precaution used to ascertain that they are entirely free from any infectious disease. A small quantity of diphtheria toxin is then injected under the skin. Usually the horse will show some reaction, such as fever and refusal to eat. Within a few days the animal becomes normal again and a somewhat larger dose of toxin is injected. These injections are repeated at intervals with increasing doses until very large quantities of toxin are injected at one time, quantities as great as 400 or 500 cc. sometimes being introduced. The immunity of the horse increases rapidly under this treatment. This process of immunization is continued for several months, then the antitoxin is secured from the blood of the horse by introducing a hollow needle or canula into the right jugular vein and allowing the blood to flow into a sterile jar. About a liter of blood can be drawn for every 100 pounds weight of the horse. The animal is allowed to regain its strength, when injections are begun again, and after a time the bleeding may be repeated. The blood is placed in a refrigerator until it has clotted and the clear straw-colored serum has separated from the fibrin and corpuscles. This is pipetted off and constitutes the antitoxin of commerce. Before being used, its strength or potency is determined by finding out how much diphtheria toxin a given amount will neutralize. This is accom-

plished by mixing definite amounts of toxin with varying amounts of antitoxin and injecting into guinea pigs. In this manner the number of *immunity units* is determined. Before being sold, it is filtered through porcelain filters to remove any bacteria that may have gained entrance during the manipulation. A small amount of preservative is also usually added. It is placed in receptacles,

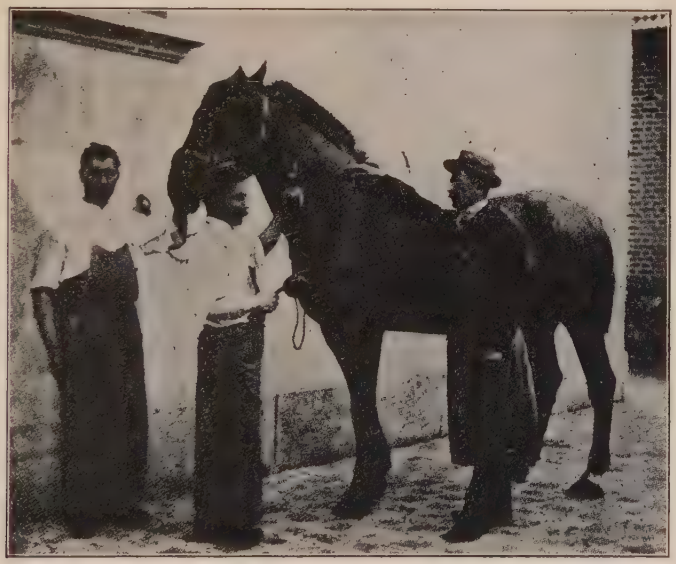


FIG. 145. — Injection of diphtheria toxin into a horse. (After Kraus and Levaditi.)

usually closed syringes, and is then ready for use. Subcutaneous injections in the human are utilized to prevent or cure diphtheria.

Antitoxins of Other Types. — Antitoxins have been prepared for all of the true bacterial toxins, but only one antitoxin other than that specific for diphtheria has come into common use. This is the antitoxin produced for the toxin of the tetanus bacillus, the cause of lockjaw. Antitoxins capable of neutralizing the venom of snakes have also been prepared and may be bought upon the market. These antitoxins are manufactured in essentially the same manner as that specific for diphtheria, though there are pro-

nounced differences in details. For example, the tetanus bacillus is an anaërobe, and the toxin must therefore be produced in the absence of free oxygen.

The Origin of Antitoxins. — It is believed that toxins harm the cells of the body because they combine with certain atom groups of the cell and injure the protoplasm. They do not unite indiscriminately with any cell. For example, the toxin of the tetanus bacillus usually unites only with the cells of the nervous system. Probably these nerve cells are the only ones which contain atom groups or chemical compounds with which the toxin can combine. These atom groups are probably parts of the molecules of the proteins of the protoplasm. They are termed *receptors* (or *side chains*, whence the designation *side-chain theory*). It has been urged that these receptors are normally useful in fixing molecules of food to the protoplasm, and that their union with a toxin is a diversion from their normal function. It can be demonstrated that after toxins have united with some of these cell receptors, but in quantities insufficient to cause the death of the cell, the latter responds by producing a greater number of this same kind of cell receptor. This is in accordance with the phenomenon which occurs when tissues of any part of the body are injured or irritated. Constant friction of the skin on the hand, for example, results in the development of a callus at that point, an overproduction of skin in response to irritation. It is believed that the new cell receptors increase in number in much the same manner. In fact, they increase to such

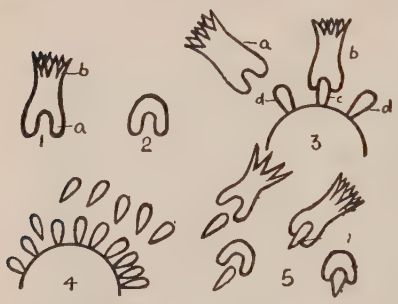


FIG. 146. — Diagrammatic representation of the formation of antitoxins. 1, a molecule of toxin, with haptophore or binding group (*a*) and toxophore or poisoning group (*b*). 2, toxoid, a toxin molecule that has lost its toxophore. 3, a protoplasmic molecule with receptors (*d*). The toxin molecule unites with the receptor as represented at (*c*.) 4, a cell which has produced an excess of receptors and is throwing these off as antitoxin molecules. 5, union of toxin and antitoxin and of toxoid and antitoxin.

numbers that many of them are thrown free into the cell plasma and eventually gain entrance to the blood stream. These freed cell receptors still retain their power to unite with molecules of toxin. In short, they constitute the antitoxin molecules. When one of these antitoxin molecules neutralizes a molecule of toxin, the latter is no longer able to unite with a cell and to injure it, somewhat as an acid once neutralized by an alkali can no longer combine with more alkali when this is added.

Agglutination and Agglutinins. — It was discovered by Gruber and by Widal that when a little of the blood serum from an individual suffering from typhoid fever is introduced into a young broth culture or suspension of typhoid bacilli, these organisms rapidly clump together and settle to the bottom of the tube. When the process is watched under the microscope, the bacteria are observed gradually to lose their power of motility, and to gather in clusters or clumps. These bacteria are not destroyed by this process, for when placed upon the surface of suitable culture medium, they will be found to develop normally. A similar phenomenon has since been noted in the blood of animals and man affected with diseases other than typhoid fever. Blood serum from a person that has dysentery, for example, will clump or agglutinate the dysentery bacilli, that from one having Malta fever will clump the bacilli characteristic of that disease. The reaction, therefore, is said to be *specific*, for the blood serum from a person that has a particular disease will clump only the organism which causes that disease.

These facts have been of considerable use in the diagnosis of disease. When a physician wishes to determine whether or not a patient has typhoid fever, he is greatly aided in his diagnosis by the results obtained from the *agglutination* or *Widal* test. He draws a drop or two of blood from the tip of the finger or the lobe of the ear, carries it to his laboratory, and there mixes the serum with a suspension of typhoid bacilli in dilutions of 1–20 and 1–40. The reaction is observed under the microscope, and if the characteristic clumping occurs in the course of half an hour, the diagnosis is positive for typhoid fever. This means cannot be used for diagnosing all diseases, however, for in some cases specific agglutinating substances are not produced in the blood, and in other cases the

difficulty of getting a homogeneous suspension of the bacteria renders the method impracticable.

The substances developed in the blood capable of causing agglutination are called *agglutinins*. They may be developed in laboratory animals by the systematic injection of killed or living cultures of the bacteria for which agglutinins are desired.

Just what agglutinins are, and how they bring about clumping is a problem which can be answered only by a study of certain

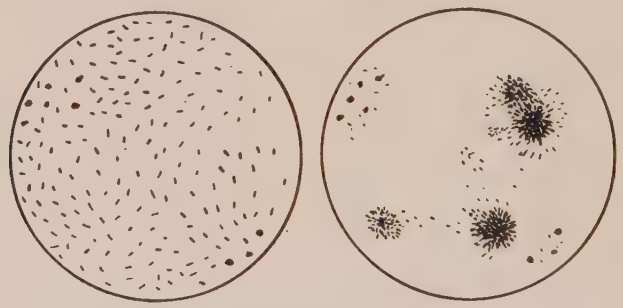


FIG. 147. — Microscopic agglutination or Widal test. *a*, bacteria of the check showing the cells uniformly distributed over the field. *b*, after the addition of blood serum containing agglutinin the cells clump in the fashion indicated.

facts which have been developed in the field of colloidal chemistry. The flocculation of colloidal suspensions has received intensive study in recent years.

A suspension of bacterial cells in many respects behaves as does a true colloidal suspension. One of the factors which tends to keep particles in suspension is the electrical charge which they carry. Bacterial cells in suspension may be shown usually to carry such charge, and inasmuch as all are charged alike, they tend to repel each other. On the other hand the bacterial cells have some tendency to stick to each other, or cohere. Whether or not they clump is determined in large part by the relative strength of the electrical repulsive force on the one hand and the cohesiveness of the cells on the other. Many substances are known which when added to a suspension may alter the charge on the particles, or alter the cohesiveness. Among such substances are certain salts.

Apparently the specific action of the agglutinin is to unite with

the bacterial cell and change its sensitiveness to salts, particularly to sodium chloride. If agglutinin and bacteria (both salt free) are mixed, agglutination does not occur until some salt is added. One may consider therefore that agglutinin specifically sensitizes bacteria so that they are agglutinated by salt action. There is a change both in charge and cohesiveness so that the latter is in the ascendency. The bacteria which are agglutinated need not be alive, for a suspension of killed organisms will agglutinate quite as readily as those which are living.

Just what advantage these agglutinins may be to the body is not certain. They are undoubtedly a part of the general mechanism of immunity, but the exact function they perform is not entirely clear. Their usefulness in the laboratory is, however, very definite. They are utilized in the following ways.

1. *Disease Diagnosis and Differentiation.* — It has been found possible to diagnose some diseases, such as typhoid, as noted above. It is also practicable to differentiate cases of paratyphoid from those of typhoid which are clinically somewhat similar.

2. *Differentiation of Bacteria.* — It is possible to identify bacteria by use of the appropriate homologous serum. For example, if one has isolated a number of pure cultures of bacteria from contaminated water, it is comparatively easy to determine which are cultures of typhoid bacilli by use of a serum known to contain agglutinins specific for typhoid bacteria.

Precipitation and Precipitins. — We have noted above that the injection of *suspensions* of bacteria into an animal will cause the animal to produce agglutinins specific for that organism. It has also been determined that the injection of proteins in *solution* will cause the formation in the animal body of similar substances which will *precipitate* these proteins. If, for example, a dilute solution of egg albumin is injected at intervals of a few days into a rabbit, and a few drops of the blood of that rabbit then drawn and the serum added to a solution of the albumin, a precipitate will be formed. The phenomenon differs from that of agglutination in that in the latter the bacteria are in suspension, in the former, a protein is in solution. The antibody developed capable of causing precipitation is termed a *precipitin*.

This precipitation phenomenon is utilized in several ways. The reaction is very specific; that is, the blood serum of an animal immunized against one kind of protein will cause precipitation of that particular type of protein alone. The protein of muscle of two different species is so similar chemically that it would be difficult if not impossible for the chemist to detect a difference. By means of the precipitation reaction, however, differences may be found readily.

This test is used in certain European countries where horseflesh is commonly sold for food as a means of differentiating between this and beef. The sale of horseflesh is permitted by law, provided that it is not sold as beef. There are certain chemical differences between the fat of horseflesh, and that of beef, and in the fresh tissues there are some differences in the amount of glycogen present, but these differences are not readily detected, and chemical analyses are not always reliable. The laboratory whose duty it is to supervise this kind of meat inspection keeps on hand several animals, usually rabbits, which have been immunized by repeated injections of the juices from horseflesh, and others immunized against beef and other flesh. A sample of the meat suspected of being horseflesh is brought to the laboratory, minced, extracted with water and the juice pressed out. Various dilutions of blood serum from the animal immunized against horseflesh are then added to the samples of the extract. If the meat is horseflesh, a precipitate will be produced. If not, there will be none. In the latter case, a test may be made using the blood serum from the animal immunized against beef, and the precipitate should develop. This method may also be used to differentiate the various meats that are used in the preparation of sausage. An extract made from sausage should be precipitated by the serum from animals immunized against each of the kinds of meats used in that sausage. Such determinations are difficult if not impossible by chemical or microscopic means.

Another use which has been found for the precipitation reaction is that of the determination of the origin of blood stains. It is sometimes necessary in legal procedure to determine whether a particular blood stain is of human or animal origin. Rabbits or

other laboratory animals are systematically immunized against human blood by repeated injections at intervals of a few days. The blood serum from such animals is then tested by mixing with serum from human blood, when a precipitate should be produced. The blood stain in question may then be tested by dissolving the serum in physiological salt solution, adding some of the serum from the immunized animal, when a precipitate will form if the blood is of human origin but not if of animal origin.

Just what part the precipitation phenomenon plays in the protection of the body against disease is quite uncertain. It is probably very closely related to the phenomenon of agglutination.

Cytotoxins and Cytolysins. — The injection of cells foreign to a given individual into the blood or tissues of that individual will incite these tissues to the production of substances capable of destroying or dissolving the injected cells. For example, if dead typhoid bacteria are injected into a rabbit repeatedly and in increasing doses and this followed by injections of living bacteria, the blood of the animal gradually acquires the ability to kill and even partially to dissolve typhoid bacilli. This may be demonstrated by adding the blood serum from this rabbit in sufficient quantities to a suspension of living typhoid bacilli. Cultures made from this suspension at intervals will show that the bacteria are rapidly destroyed. This reaction may be secured not only with bacterial cells but with cells of other kinds. If the red blood corpuscles of one species of animal are injected into the blood stream of another, the blood serum of this animal acquires the property of dissolving the red blood corpuscles of the first. This general phenomenon of the destruction of cells is termed *cytolysis*, and the substances present in the serum capable of bringing about this destruction are termed *cytotoxins* or *cytolysins*. A cytolysin which will destroy bacteria is termed a *bacteriolysin*, one which will destroy red blood corpuscles a *hemolysin*, etc.

Bacteriolysins are undoubtedly of considerable importance in the development of immunity to disease. The blood serum of a person who has recovered recently from typhoid and who is, therefore, relatively immune to the disease, will be found to destroy typhoid bacteria. In fact, many of the non-pathogenic bacteria,

and even some of those which are commonly pathogenic are destroyed by bacteriolysins found normally in the healthy body. When one becomes immune to a disease or develops an active immunity in many cases the amount of this bacteriolysin is considerably increased. This furnishes an efficient barrier to the development of organisms in the body.

Inasmuch as bacteriolysins can be developed for a considerable number of bacteria by injection of these organisms in increasing doses into suitable animals, it is possible to produce *bacteriolytic* or so-called *antibacterial sera*, in a manner analogous to that used in the production of antitoxin. Usually large animals, such as the goat or the horse, are used for this purpose. The bacteria are grown upon culture media and injected in increasing doses into the animal to be immunized. If this animal is susceptible to the particular disease caused by this microörganism, it is sometimes necessary to begin the injections with dead bacteria, and when a certain degree of resistance or immunity has been developed, to follow this with injections of the living bacteria. The blood is then drawn from the animal and the serum collected, as has been described for diphtheria antitoxin. This serum, however, does not contain antitoxin, but bacteriolysin. It is termed an *antibacterial serum*, a *bacteriolytic serum*, or simply an *antiserum*. Unfortunately, bacteriolytic sera have not proved efficient in most diseases. There are a few exceptions to this. Cerebrospinal meningitis in man, for example, is best treated by the use of a specific antiserum. One of the difficulties, perhaps, is the fact that the bacteriolysin developed in the body of one species of animal is not always efficient when introduced into the body of another species.

Bacteriolysins are not simply constituted, as are the antitoxins, but may be shown to be made up of two substances; one termed *complement* is present in most normal blood, the other called *amboceptor* is produced as a result of immunization. The complement is readily destroyed by heat; 56° C. for thirty minutes will render it inactive. A bacteriolytic serum which is thus heated will lose its power to destroy bacteria. This power is also lost after the serum has been allowed to stand for a time in contact with air or in a warm place. Such a serum is said to be *inacti-*

vated. It is found that this serum, however, can be made active again, that is, capable of once more destroying bacteria, by the addition of a little normal serum. In other words, neither normal serum nor the inactivated serum alone can destroy bacteria, but when the two are mixed, such destruction is possible. The nature of the reaction is, therefore, much more complex than is the case with antitoxins and toxins. It is believed that the amboceptor first unites with the bacterial cell and sensitizes it. The complement is then able to unite with the bacterial cell by means of the amboceptor and can destroy the organism. The complexity of the reaction and the instability of the complement explain in some measure the fact that antisera are used in the treatment of comparatively few diseases.

Hemolysins have been studied in the laboratory even more than have the bacteriolysins. This is in part because of the ease with which the destruction of red blood corpuscles may be observed. Experimental work may be carried on with red blood corpuscles from any one of several species of animals. Probably those from the sheep are most commonly used. For production of a hemolytic antiserum the rabbit is most frequently employed. The blood from the sheep is either defibrinated or mixed with a solution of sodium citrate to prevent coagulation. It is then centrifugated and the clear supernatant serum removed from the sedimented red blood cells. The latter are then suspended in physiological salt solution, and again centrifugated. This washing process may be repeated several times. Eventually a suspension of red blood corpuscles in physiological salt solution is secured. These are injected repeatedly at suitable intervals into a rabbit. The animal will develop hemolysins in its blood. The animal may be bled and the blood serum secured. A drop of this blood serum added to a suspension of the red blood cells of the sheep will cause the latter to go more or less completely into solution. Any cells which are not dissolved soon settle to the bottom. The supernatant liquid is more or less strongly tinged with red, the depth of color observed, depending upon the number of cells dissolved.

The development of bacteriolysins (or specific amboceptor) in the blood of an individual suffering from certain chronic diseases may

be utilized as a means of diagnosing the disease. An examination of the blood for the specific amboceptor may then constitute a satisfactory method for diagnosis. This is most easily carried out by the technique described below. The general method has been employed particularly for the recognition of glanders in the horse and a modification of it (called the Wassermann test) for the diagnosis of syphilis in man. The test is usually called the complement-fixation test for the diagnosis of disease.

The complement-fixation test is carried out by use of the following materials.

1. *Bacterial Antigen.* — This is a suspension of bacteria specific for the disease. If the disease being diagnosed is glanders, for example, this would be a suspension of glanders bacilli.

2. *Serum from the Patient.* — If this is from a horse having glanders it should contain the amboceptor specific for the glanders bacillus. It would also contain complement. The latter is destroyed by heating the serum to 56° C. for 30 minutes.

3. *Blood Serum from the Guinea Pig.* — This blood serum may come from any normal animal. It contains complement but no amboceptor.

The test is initiated by mixing suitable proportions of Nos. 1, 2, and 3. If the disease is present, the amboceptor will unite with the glanders bacilli, and the complement in turn will unite with the sensitized bacteria, that is, the complement will be *fixed* by the bacteria. If the horse is not diseased there will be no amboceptor present, and the complement will remain in solution. It is evident that a suitable reagent for detecting whether or not the complement has remained in solution is highly desirable. Such a reagent is to be found in sensitized red blood corpuscles.

4. *Sheep Red Blood Corpuscles.* — These should be washed and suspended in physiological salt solution.

5. *Blood Serum from a Rabbit* which has been immunized by repeated injections of the red blood cells of the sheep. — This is heated to 56° C. for 30 minutes to destroy the complement which is present.

Nos. 4 and 5 are mixed in suitable proportions. The amboceptor specific for the red blood cells unites with them. This mixture con-

taining the sensitized blood cells is next added to the mixture of Nos. 1, 2, and 3 noted above. It is evident that if there is any complement remaining in solution, the red blood corpuscles will be dissolved. If, on the other hand, the complement has disappeared from solution because of the presence of the glanders amboceptor the red blood cells will not be dissolved. In reading this test, positive hemolysis indicates the disease to be absent; no hemolysis indicates the presence of the disease.

Opsonins. — In the definition of Metchnikoff's theory of phagocytosis it was noted that certain of the body cells termed *phagocytes* are capable of engulfing and destroying foreign bodies such as bacterial cells under certain conditions. For the most part, these phagocytes are *leucocytes* or white blood corpuscles, although there are some of the fixed body cells which also can destroy bacteria. There have been described from normal blood of man five or six different kinds of leucocytes. Some of these are not active in the destruction of microörganisms, that is, in phagocytosis. Probably most important are the forms known as *polymorphonuclear leucocytes*. These are white cells somewhat larger than red blood corpuscles containing a nucleus which is very irregular in shape, frequently in the form of a horseshoe or separated into several more or less spherical nuclei connected by threads. Certain of the cells possessing round or spherical nuclei are also phagocytic.

It has been found that these phagocytes are not usually able to engulf and destroy microörganisms without the assistance of certain substances to be found in the blood serum. An experiment such as the following may be used to demonstrate this fact. If normal blood is drawn into a solution of sodium citrate or oxalate, coagulation will not occur. The citrated blood may be placed in a centrifuge, whirled about rapidly until the blood corpuscles are thrown to the bottom and the serum rises to the top. This blood serum may then be pipetted off and physiological salt solution added to the blood corpuscles. These are then shaken up with salt solution and again centrifuged. The supernatant liquid is removed and replaced by fresh salt solution. By repeating this process several times the corpuscles may be washed entirely free from blood serum. Careful examination of the sediment in the centrifuge tube will show

that the white blood corpuscles or leucocytes are most abundant in the surface layer of this sediment, and can be removed by means of a pipette. When these are mixed with suitable microörganisms and stained mounts made from time to time, none of the bacteria will be found within the bodies of the leucocytes. If, however, some of the blood serum is added to such a mixture, stained mounts will soon show the presence of considerable numbers of bacteria within the leucocytes. The experiment may be varied by mixing the bacteria with the serum, allowing them to stand for a time, and then washing them entirely free from the serum. When these bacteria are brought into contact with the washed leucocytes they are rapidly engulfed. It is evident, therefore, that there is something in the blood serum which so changes the bacteria that they are readily engulfed by leucocytes. The bacteria may be said to be rendered *positively chemotactic* for the leucocytes. The substance present in blood serum which brings about this change of bacteria is termed an *opsonin*. This word comes from a Greek word (*opsoneo*) meaning to set a table or prepare a meal; its appropriateness is obvious.

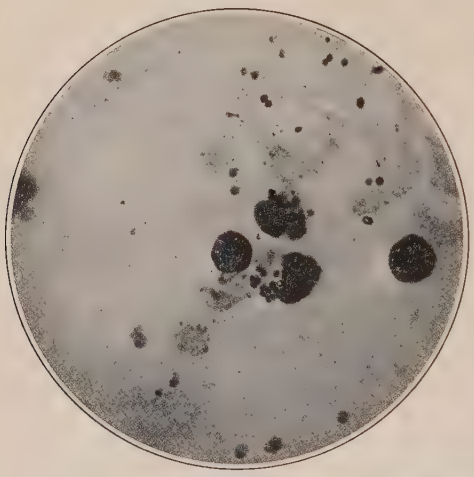


FIG. 148. — White blood corpuscles (phagocytes) with ingested bacteria. (× 1000.)

Opsonins which are capable of bringing about phagocytosis of one species of bacteria ordinarily have no effect upon another species, that is, the opsonins resemble antitoxins, agglutinins, and bacteriolysins in being specific. It is found, furthermore, that an individual that has been immunized to a certain disease contains within his blood serum a much larger proportion of opsonin than is

carried in normal or non-immune serum. In other words, immunity in some diseases is undoubtedly due to the production in the blood serum of substances (opsonins) which enable the white blood corpuscles to destroy the invading pathogenic bacteria. Methods have been evolved for determining the ratio of opsonin in the blood of such an individual to that in the blood of a normal individual. This ratio is termed the *opsonic index*. It is believed, for example, that when the opsonic index of a person that has a certain disease rises above unity, it is to be considered as a favorable symptom, as it indicates that increased phagocytosis is possible.

The opsonic content of the blood may be increased by the injection of killed or attenuated bacteria. This process of immunization is termed vaccination, and will be discussed later.

It is probable that the antisera previously discussed under the heading of bacteriolysins may owe their effectiveness in part to the presence of opsonins. In general, however, efforts to bring about the development of passive opsonic immunity have not proved particularly successful.

Anaphylaxis, Allergy, or Hypersusceptibility. — Another phenomenon which undoubtedly has much to do with the resistance of the body to disease has been much studied. It is the phenomenon of *hypersusceptibility* or *anaphylaxis*. It is a well-known fact that some individuals cannot eat certain foods; for example, strawberries. To such persons they act as poison. This is termed hypersusceptibility to the chemical compounds in the strawberries. It has been found possible to produce similar hypersusceptibility by experimental methods in the laboratory with certain animals. For example, if one injects a dilute solution of a protein such as egg albumin into the body of a guinea pig and does not repeat the injection for ten days or two weeks, a second injection will cause symptoms of poisoning. Two or three cubic centimeters of egg albumin injected intraperitoneally into such an hypersusceptible animal will kill it within a few minutes. Egg albumin is not to be considered as normally poisonous, but injections made in this manner render the body unusually or abnormally sensitive. Such an animal is said to be in a state of *anaphylaxis* toward egg albumin. It is interesting to note that this reaction is specific as are the others

which we have discussed. If a guinea pig is rendered anaphylactic toward egg albumin, the injection of some other protein, such as blood serum of the horse, will not cause any reaction. On the other hand, the guinea pig may be sensitized to practically any protein and to many of the more complex protein derivatives. The reaction known as anaphylaxis has been demonstrated in a considerable number of species of animals.

One of the most frequently observed of the anaphylactic phenomena is that of hay fever. A person may in some manner become sensitive (allergic) to the compounds present in the pollen of certain plants. Thereafter, whenever this pollen is inhaled, it may cause severe irritation of the mucous membranes of the nose and throat. Similarly, the inhalation of dust from various sources may be the cause of asthma. The sensitiveness of an individual to a particular type of protein or other foreign material may be tested by the injection of a small quantity of this material just under the skin, or by rubbing it vigorously into the skin. The tissues of a person who is sensitive will become inflamed and somewhat swollen, while those of a normal person, that is, one who is not in a condition of allergy, will exhibit no reaction.

Similarly, individuals may sometimes become sensitized to the proteins or growth products of certain bacteria. This occurs most frequently in certain chronic diseases. It is believed that the phenomenon of anaphylaxis explains certain rather obscure body reactions in such diseases. The common method of diagnosing tuberculosis in animals (particularly in cattle and to some degree in man as well) is by the injection of a small quantity of a killed culture of *Mycobacterium tuberculosis* subcutaneously into the suspected individual. An animal that has no tubercle bacilli growing in the body will show no change, but one that is tuberculous will show within a few hours a definite reaction in which the temperature rises and in the course of a day or two comes back to the normal. It is probable that the explanation of this reaction is to be sought in anaphylaxis. The presence of the tubercle bacilli in the body has sensitized the body to the products of this organism and the injection of the dead tubercle bacilli, or *tuberculin* as the material is called, causes on the part of the body

a reaction of the same general nature as that which is produced in the guinea pig by the second injection of the protein, or by the injection of the suitable pollen into one who has hay fever.

This method of diagnosis has also been used in certain other diseases, as in glanders of the horse.

Vaccines and Vaccination. — A vaccine is a killed or weakened (attenuated) suspension of organisms inoculated into the body for the purpose of causing the development of an active immunity. It is, of course, not ordinarily desirable to inject virulent micro-organisms, but by the use of dead or attenuated forms, a reaction or mild attack of the disease may be produced, followed by a development of immunity. Vaccines consisting of dead bacteria are usually termed *bacterins*. These bacterins are utilized to some extent in immunization against chronic skin eruptions, as in acne, and in the treatment of boils and chronic erysipelas. Typhoid vaccine is a vaccine prepared by suspending in physiological salt solution typhoid bacilli grown upon an agar culture and heating until the bacteria are killed. When injected into the body this material causes the development of an active immunity toward typhoid fever. The vaccine commonly used in smallpox is obtained from the lymph produced on the skin of calves by inoculation with attenuated smallpox virus. The organism causing smallpox has never been isolated and grown in pure cultures, but it is known to be present in the lymph found in the pustules which develop upon the skin in this disease. This lymph is used for inoculating the calf and the lymph from the vesicles of the calf is dried upon bone points or mixed with glycerol and sealed hermetically in capillary tubes. This is then used in the production of a mild infection in the human, and as a result of this infection, a considerable degree of immunity to smallpox is developed.

It will be noted that the immunity conferred by the use of vaccines is an active acquired immunity. It may be due in different cases to the development in the body of bacteriolysins or opsonins, or possibly some other substances which have not as yet been described or identified.

Bacteriophage. — It is occasionally observed that in certain cultures of bacteria the medium will suddenly clear and few or no

living bacteria will remain. This phenomenon of sudden destruction or lysis of bacteria was noted by Twort and by d'Herelle. The phenomenon was studied by the latter investigator in cultures of dysentery bacilli. He found that if fecal material was filtered through a porcelain filter so fine that bacteria did not pass, a mixture of a small amount of the filtrate with a young culture of dysentery bacilli would cause the latter to be more or less completely dissolved. The reaction was so striking that it has been studied intensively by many investigators in recent years. There is no question but that there is a relatively powerful agent capable of producing bacteriolysis under the right conditions. There are, however, two distinct schools of thought as to the nature of this material.

One group of investigators, following the lead of d'Herelle, believe that this lytic principle, termed *bacteriophage*, is an ultramicroscopic organism which parasitizes the bacteria. In fact, d'Herelle gave to this organism, which he believed he had demonstrated, the name *Bacteriophagum intestinale*. He concluded that this minute organism (*filterable virus*) grows on the bodies of the bacteria and destroys them, in fact, produces a fatal disease of the bacteria in a fashion analogous to the production of a disease in a higher form by bacteria. When a suspension of bacteria thus parasitized is filtered, the filtrate is found to be able to induce lysis in other bacterial cultures of the same species into which it is introduced. He has brought together many arguments in favor of this theory as to the nature of the bacteriophage.

Another group of investigators is equally convinced that the bacteriophage is not an ultramicroscopic organism but is something of the nature of an enzyme. They believe that the bacteria when attacked by this enzyme are destroyed, and in the process of destruction increased quantities of the destructive (lytic) material are liberated. According to these individuals bacteriophage is not a living cell, although in many cases it is regarded as being particulate. It is believed by some investigators that the destruction of the bacteriophage is quite possibly due to an over-stimulation of the bacteria. Bronfenbrenner, for example, has shown that they apparently disintegrate suddenly, almost explosively, and as

though some stimulus had produced a high internal pressure in the cells, with consequent rupture.

Inasmuch as bacteriophage is non-toxic to man and animals it has been suggested that it might be utilized in the treatment and cure of disease. Many efforts have been made in this direction, and some favorable reports of this method are at hand. However, the evidence in favor of such utilization of bacteriophage is not particularly convincing at the present time.

Summary. — A perusal of the contents of this chapter will indicate that immunity in the body may be due to one of several causes. In certain diseases, particularly in diphtheria and tetanus or lockjaw, *antitoxins* are developed which neutralize the toxins of the organisms causing the diseases. In some diseases antitoxins may be used both for prevention and for cure. *Bacteriolysins* capable of dissolving pathogenic bacteria specific for certain diseases are sometimes developed, and account for immunity in many cases. In a few instances bacterial sera produced in the body of animals may be used for the treatment and prevention of disease. A third type of immunity may be conferred by the *opsonins* which enable the white blood corpuscles to destroy certain bacteria which may gain entrance to the body. For the stimulation of opsonin production in the body, vaccines are commonly used. In addition to these substances which determine immunity, the body may also produce *agglutinins* specific for bacteria and useful in diagnosing diseases, and *precipitins* commonly used in the differentiation and identification of proteins.

CHAPTER XXXIV

MICROÖRGANISMS NORMAL TO THE HUMAN BODY

Many species of bacteria are known which develop on the skin and in the various body cavities which open to the surface. The tissues of the body are normally sterile. Bacteria are not present in the blood stream, the muscles, or glands of normal individuals, although the lymph nodes lying near the intestines may occasionally be found to be infected by intestinal bacteria. This probably comes about through the organisms finding their way through the intestinal wall and then being carried by the lymph to the lymph glands. It is probable that they are there destroyed.

Organisms may or may not be present in the tissues of diseased individuals. It has been noted that some diseases are classed as septicemias and bacteremias because of the general distribution of the organisms through the body in the blood stream. In other cases the organisms are localized and do not get far from the original site of infection.

The exposed surfaces of the body and the cavities that communicate with the surface may be said to possess a normal bacterial flora. The organisms constituting this flora are worthy of consideration, for although they do not usually produce disease, some of them under unusual conditions may do so.

Flora of the Skin. — Two groups of skin microörganisms may be differentiated, those which are merely accidental and those which are quite constantly present and evidently multiply upon the body surfaces. Whenever the skin comes in contact with dirt or dust, or any object coated with bacteria or containing them, organisms may adhere. The bacteria of the soil are quite common upon the skin, particularly under the finger nails and in the hair. These include such aërobic forms as the organisms belonging to

the hay bacillus or *Bacillus subtilis* group, and certain anaërobic bacteria. Organisms characteristic of the intestines, such as *Bacterium coli*, are also commonly present. Winslow, for example, has shown *Bact. coli* to be present on the hands of ten individuals out of one hundred and eleven examined. Pathogenic bacteria may be present upon the skin of one who comes in contact with diseased individuals. Tubercle bacilli may be found, for example, upon the hands of those employed in tubercular wards of hospitals or who care for tubercular patients in the home. The most common normal inhabitant of the skin, and one which penetrates to the lower layers of the epidermis, is the *Staphylococcus epidermidis albus*, an organism very closely related to the pus-producing cocci. *Staphylococcus aureus* and *Staph. albus* are not uncommonly present, but usually are lacking in virulence. These are the organisms found in wound infections, abscesses, and boils. Streptococci are also frequently isolated from the skin, though more rarely than the preceding. Wherever the skin is continually moist and greasy, as in the axillæ of the body, *Mycobacterium smegmatis*, an acid-fast organism closely resembling *Mycobacterium tuberculosis*, has been described as constantly present. This organism does not seem to penetrate the skin, but to live in the skin excretions. The *Micrococcus cereus flavus* is generally found in the wax secreted in the external auditory canal. The exposed mucous membrane of the conjunctiva shows relatively few bacteria in health. The secretion of tears seems to remove and perhaps also to destroy organisms that may accidentally gain entrance.

The Flora of the Mouth. — A larger number of bacteria have been described from the mouth. Many of these forms doubtless are accidental, but the normal mouth has a constant flora consisting of a considerable number of species. These were first studied extensively by Müller, who decided six organisms to be practically always present. These organisms were in part relatively long threads (*Leptotrichia*) and slender spirilla (*Spirochæta*). He did not succeed in cultivating any of these. Spirochetes from the mouth, particularly the *Spirochæta dentium* of Müller, have been cultivated by Noguchi by the same method earlier described for the cultivation of the organism specific for syphilis (*Treponema*

pallidum). These organisms are obligate anaërobes when grown in culture media, but find favorable conditions for growth between the teeth and in teeth that are decaying. They are actively motile and are frequently to be observed moving about rapidly when a little of the tartar or material from between the teeth or lying between the gums and the teeth is suspended in physiological salt solution and examined under the microscope. Some five or six species or spirochetes have been recognized as relatively common. Certain anaërobic bacilli, some of them related to the *Clostridium putrificum* already described, have been noted in decaying teeth. Among the cocci, certain streptococci, in large part non-virulent varieties of *Streptococcus pyogenes* are almost always present. The same is true of *Staphylococcus aureus* and *Staph. albus*. *Diplococcus pneumoniæ* or the pneumococcus can frequently be observed in the stained mounts of the sputum of healthy individuals. Such organisms probably are avirulent forms in most cases. The decay of teeth has been ascribed to the development of acids by some of the lactic acid bacteria which may be present upon the surfaces. This results in the more or less complete removal of the lime present, while the decomposition of the remaining substances of the teeth probably is brought about by common anaërobic bacteria. A foul breath is usually due to lack of careful cleaning of the teeth and mouth. The putrefactive bacteria bring about decomposition of particles of food retained within the mouth and caught between the teeth.

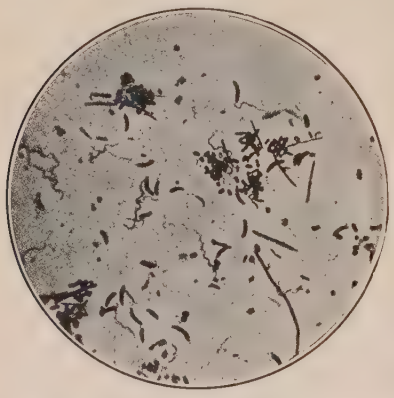


FIG. 149. — Bacteria from the tartar of the teeth. (After Günther.)

The saliva seems to exert very little bactericidal action. The breath, except when forcibly expired as in sneezing and coughing, is free from bacteria as it leaves the mouth or nose.

Occasionally the mouth of a healthy individual may harbor (as in a bacillus carrier) some of the disease-producing organisms. Most important of these are the *Corynebacterium diphtheriæ* and *Streptococcus scarlatinæ*, the specific causes of diphtheria and scarlet fever respectively.

Flora of the Alimentary Tract. — The alimentary tract is sterile at birth, but a few hours later bacteria are found to be present. Whether or not animals can continue to live without organisms in the intestines is a disputed point. The feces of animals living in polar regions have been shown to be almost entirely devoid of bacteria. Young guinea pigs removed by Cæsarian section from the mother have been kept alive for considerable time under sterile conditions. On the other hand, an effort to raise chicks without bacterial infection has resulted in failure. It is not improbable that bacteria may be essential to health in some animals, but many species can maintain themselves very well without the presence of any organisms at all in the alimentary tract.

Stomach. — Bacteria do not normally develop in the stomach, owing to the acidity of the gastric juice. When this acidity is diminished for any reason, bacteria may multiply. Organisms belonging to the butyric acid group and *Bacterium aërogenes* may produce an active fermentation of the food taken into the stomach with the development of gas and other products of decomposition. In certain conditions of the stomach, lactic acid bacteria of the *Lactobacillus bulgaricus* type have also been found.

Intestines. — Bacteria are relatively sparse in the first portion of the small intestine (the duodenum). As the food passes along the intestines, however, the acidity is neutralized and conditions are rendered favorable for bacterial growth. Certain of the digestive juices, particularly bile, are markedly antiseptic toward some microörganisms and more or less completely inhibit their growth. This is not true, however, of the normal flora of the intestines. In the lower part of the small intestines, or ilium, bacteria become more numerous. *Bacterium aërogenes* is present and *Bacterium coli* in smaller numbers. In the colon the bacteria reach their maximum development. *Bact. aërogenes* and *Bact. coli* are practically always present, the latter in great numbers.

These gram negative forms in addition to the gram positive *Lactobacillus bifidus* and *Lact. acidophilus* and related forms are believed to inhibit the growth of many other species, particularly the putrefactive forms, by the formation of acid and possibly of other metabolic products. Certain anaërobic spore-producing forms, particularly *Clostridium putrificum* and *Cl. welchii* already described, are also quite constantly present. They rarely gain the

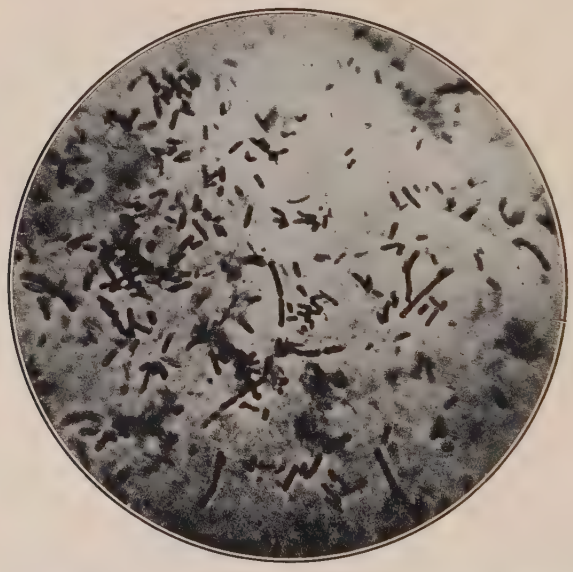


FIG. 150. — Stained mount from human feces, showing types of bacteria present. (× 1000.)

ascendancy in the healthy normal intestinal tract. It is probable that in some of the herbivorous animals organisms which are capable of hydrolyzing cellulose may be of assistance in the digestion of the food. Such cellulose-digesting organisms have been isolated from the feces of the horse. The alimentary tracts of certain insects, as the termites or white ants, contain protozoa which are apparently essential to the digestion of the cellulose and wood ingested. Protozoa in the rumen of cattle have been claimed to be of significance in digestion, but the proof is inadequate.

Becker has shown that goats may assimilate food normally without these organisms being present.

Human feces contain relatively enormous numbers of bacteria. Frequently from one-fourth to one-third of the dry weight of the fecal material consists of the bodies of microörganisms. By no means all of these will develop upon artificial media. Probably in part they have been destroyed by their own products of metabolism, and in part they do not develop because suitable culture media are not used. There are usually present in each gram of human fecal material between one million and forty million living bacteria which will develop upon artificial media. The changes due to putrefactive bacteria in the intestines have been emphasized within recent years as being of considerable importance. It is believed that the products of decomposition, when these organisms are in the ascendancy in the colon, are absorbed in part through the intestinal wall and appear in the same form or as related compounds in the urine. It is believed that some of the changes characteristic of old age are due to these putrefactive substances. Principal among these changes is the hardening of the vessel walls of the arteries (arteriosclerosis). In those who show evidences of old age, these putrefactive bacteria have commonly been found to constitute a major portion of the intestinal flora. Metchnikoff and his coworkers have believed that these putrefactive organisms may be supplanted in the colon as a result of continued use of foods or beverages containing large numbers of lactic acid bacteria. The putrefactive forms do not develop in the presence of lactic acid or of the lactic acid bacteria. The organism which was advocated for this purpose was the *Lactobacillus bulgaricus*, the specific cause of the souring characteristic of Bulgarian soured milk or joghurt. The *Lactobacillus acidophilus* has been found to be better adapted to this purpose. Enough evidence has been gathered to emphasize the need of a maintenance of a proper bacterial flora in the intestines, particularly in the colon, if the general health of the body is to be conserved. That the character of this flora may be determined by the diet seems to be demonstrated.

Flora of the Respiratory Tract. — The nose is an efficient instrument for catching dust particles, bacteria, etc., which may be

present in the inspired air. The nose can scarcely be said to have a characteristic flora, but the organisms present in the air may be usually isolated from it. By the time the air reaches the bronchi, it is largely freed from organisms and only rarely do such penetrate to the bronchioles and alveoli of the lungs. These latter tissues are usually entirely free from organisms. Those particles of dust and those microörganisms that fall upon the surface of the mucous membranes are caught by the sticky mucus which is secreted and forced to pass in the direction of the mouth by the ciliated epithelium. This constitutes a kind of drainage system for the elimination of all such materials.

Flora of the Genito-urinary System. — The exposed mucous surfaces of the urinary meatus and of the vagina usually harbor certain bacteria. For the most part these are harmless, but cocci identical with the pus-producing cocci or at least closely related are commonly present. The secretions of the vagina are believed to be somewhat bactericidal, but certain bacteria are found quite constantly. It has been claimed that the characteristic properties of this secretion are in part due to the organisms present.

Bacteria do not normally develop within the uterus, although they are not uncommon in the later stages of pregnancy. The urinary bladder is normally sterile.

In the smegma about the external genitals acid-fast bacteria, particularly *Mycobacterium smegmatis*, are quite constantly present. This latter must be carefully differentiated by the diagnostician from the bacillus of tuberculosis, which it closely resembles.

CHAPTER XXXV

CLASSIFICATION OF DISEASE-PRODUCING ORGANISMS INTO GROUPS

It is convenient in a study of organisms which are capable of producing disease to bring together related forms or to classify together related diseases. The former may be termed a bacteriological and the latter a pathological classification. In a *bacteriological classification* the closely related organisms are grouped together. For example, we speak of the intestinal group of organisms which includes all of those bacteria which have certain common morphological and cultural characters and which differ among themselves in minor ways. A *pathological classification* disregards the relationships of the *organisms* and classifies together those *diseases* which resemble each other. For example, all diseases in which toxins are produced and the organisms remain localized (toxemias) may be classified together regardless of the organisms causing these various infections. Or again, all organisms capable of producing nodules or tubercles in the tissues are sometimes grouped together with the *Mycobacterium tuberculosis* under a single heading in spite of the fact that the organisms themselves are not closely related. For our present purpose the bacteriological classification will prove the more satisfactory and will be followed here.

Five principal groups of organisms will be considered, the pathogenic bacteria, yeasts, molds, protozoa, and the ultra-microscopic and filterable viruses. In addition, there are several diseases the causes of which are not known. These may constitute a sixth group.

Inasmuch as we are here interested principally in the organisms capable of producing disease in man and animals and having in consequence considerable economic importance, many of the sub-

groups commonly discussed under the heading of pathogenic bacteria may be eliminated. The groups to be discussed may be differentiated by the following key.

Key to the Principal Groups of Pathogenic Bacteria

a. Cocci
- *b.* Producing non-specific infections — 1. *Group of non-specific cocci*
- *bb.* Producing specific infections . . — 2. *Group of specific cocci*

aa. Cells elongated, not spherical
- *b.* Straight rods, not filamentous
  - *c.* Not producing spores
    - *d.* Cells not acid-fast
      - *e.* Organisms not hemophilic, *i.e.*, requiring blood or serum for culture
        - *f.* Organisms gram positive . . . . . 3. *Diphtheria group*
        - *ff.* Organisms gram negative . . . . . 4. *Plague group*; 5. *Intestinal or colon-typhoid group*; 6. *Undulant-fever-abortion group*; 7. *Glanders group*
      - *ee.* Organisms hemophilic . . . . . 8. *Hemophilic group*
    - *dd.* Cells acid-fast . . . . 9. *Tuberculosis group*
  - *cc.* Producing spores
    - *d.* Aërobic . . . . . 10. *Anthrax group*
    - *dd.* Anaërobic . . . . . 11. *Anaërobic spore-bearing group*
- *bb.* Cells bent or spiral
  - *c.* Relatively short cells . . . 12. *Vibrio group*
  - *cc.* Cells very slender, elongate 13. *Spirochete group*
- *bbb.* Cells filamentous . . . . . 14. *Actinomycetes group*

Non-specific Coccus Group. — The non-specific cocci belong to the genera *Staphylococcus* and *Streptococcus*. They are organisms not uncommon upon the surface of the skin and produce such non-specific infections as suppuration (pus production) in wounds, boils, and abscesses, and in some cases septicemia (blood poisoning).

Specific Coccus Group. — Several organisms belonging to the genera Streptococcus, Diplococcus and Neisseria produce specific infectious diseases in man. Among these are *Diplococcus pneumoniæ*, producing pneumonia; *Neisseria meningitidis*, the cause of epidemic cerebrospinal meningitis; and *Neisseria gonorrhœæ*, causing gonorrhea.

Diphtheria Group. — The principal organism belonging to this group is the *Corynebacterium diphtheriæ*, which causes diphtheria in man.

Plague Group. — Several diseases termed hemorrhagic septicemias are caused by organisms which belong to this group. The bacteria belong to the genus Pasteurella. Among the diseases are fowl cholera and the hemorrhagic septicemias of cattle, swine, horses, and other animals. The only representatives of this group which definitely produce disease in man are the *Pasteurella pestis*, causing plague in rodents and man, and *Pasteurella tularensis*, producing tularemia in rabbits and man.

Colon-typhoid or Intestinal Group of Bacteria. — This group includes a considerable number of microörganisms, both pathogenic and non-pathogenic. Many of these are found in the intestines in man and animals in health or in disease. A few are found in soil, water, and in some cases in diseases other than those of intestinal origin. The bacteria belonging to this group are frequently distributed among several genera. They will, however, be here grouped in the genus Bacterium. The most important organisms to be considered are *Bacterium coli*, of the intestine, *Bacterium paratyphosum*, the cause of paratyphoid fever, *Bacterium enteritidis*, commonly associated with meat poisoning, *Bacterium pullorum*, the cause of bacillary white diarrhea in chicks, *Bacterium typhosum*, the cause of typhoid fever, *Bacterium dysenteriæ*, the cause of bacillary dysentery, *Bacterium abortus*, the cause of infectious

abortion in cattle and other animals, and *Bacterium melitensis*, the cause of undulant or Malta fever.

Glanders Group. — A single organism is included here, the *Pfeifferella mallei*, the cause of the disease glanders in the horse, a disease also transmissible to man.

Hemophilic Group. — These bacteria require the presence of blood serum or hemoglobin in the medium in which they develop. This group includes several organisms more or less doubtfully associated with disease, including *Hemophilus influenzæ* in influenza, and *Hemophilus pertussis* in whooping cough.

Tuberculosis or Acid-fast Group. — Two organisms producing disease in man are to be included in this group, the *Mycobacterium tuberculosis*, the cause of tuberculosis, and *Mycobacterium lepræ*, the cause of leprosy. Here is also to be included the *Mycobacterium paratuberculosis*, causing a disease in cattle.

Anthrax Group. — This includes the *Bacillus anthracis*, the cause of the disease anthrax in man and animals.

Anaërobic Spore-bearing Group. — Several species of spore-bearing anaërobes belonging to the genus Clostridium have been described as producing disease in man or animals. Of particular importance are *Clostridium tetani*, the cause of tetanus or lockjaw, *Clostridium botulinum*, one of the causes of food poisoning, and several related organisms producing diseases in man and animals as a result of wound invasion.

Vibrio Group. — The only organism of economic importance belonging to this group is the *Vibrio choleræ*, the cause of the disease Asiatic cholera in man.

Spirochete Group. — The most important of the species is the *Spirochæta pallidum*, which produces the disease syphilis in man. A number of other members of this group produce other diseases in man or in animals.

Actinomycetes Group. — These organisms belong to the genus Actinomyces, and include the so-called pathogenic ray fungi or ray bacteria.

Pathogenic Yeasts and Molds. — Several species or organisms closely related morphologically to the yeasts have been described as producing disease in man, although such diseases are relatively

uncommon. A considerable number of species of molds are also known to produce disease in man. Most of these latter diseases are infections of the skin, such as ringworm.

Pathogenic Protozoa. — Among the commoner diseases produced by protozoa are dysentery, caused by *Entamœba*, and malaria, caused by *Plasmodium*.

Ultra Microscopic Viruses and Diseases of Unknown Etiology. — There is a considerable list of diseases which have been shown within recent years to be due to organisms which will pass through the pores of a porcelain filter. Among these are yellow fever, spotted fever, and certain others of the exanthemata.

CHAPTER XXXVI

THE GROUP OF NON-SPECIFIC PYOGENIC COCCI

The organisms belonging to this group are all associated with a considerable variety of infections, none of them specific in nature. They are found as the cause of pus production in wounds, boils, carbuncles, abscesses, and in inflammations of the skin, mucous membranes, and underlying tissues, such as erysipelas and tonsillitis. Occasionally they gain entrance to the circulation, and may produce septicemia by multiplication in the blood stream itself.

Many species of bacteria have been described belonging to this group. The most important of these are *Staphylococcus aureus*, *Staphylococcus albus*, and *Streptococcus pyogenes*, with the closely related *Streptococcus scarlatinæ*. These all resemble each other in that they are cocci, do not produce spores, are non-motile, gram positive, aërobic and facultative anaërobic, and grow upon most of the common laboratory media. They are differentiated from each other in that the cells of Staphylococcus are irregularly massed or grouped while those of Streptococcus occur in chains. *Staphylococcus albus* is differentiated from *Staphylococcus aureus* in that the latter produces a golden yellow pigment when grown upon laboratory media, and the former is white.

It was noted above that these organisms are chiefly responsible for pus production. Organisms of this type are said to be *pyogenic*, and the process of pus production is termed *suppuration*. When these organisms gain entrance to the body (this is true also of other organisms) they produce an inflammation. They begin to destroy and to some extent to disintegrate the tissues with which they are in contact. Substances more or less poisonous in nature are produced, possibly in part the results of synthetic action of the

organism, but probably in greater part, the products of the breaking down of the tissue cells. The blood vessels in the surrounding tissues become engorged with blood. Blood serum leaves the vessels and infiltrates these tissues, usually causing swelling. The white blood corpuscles also leave the capillaries in large numbers and pass out among the tissue cells. In the immediate vicinity of the infected tissues, they form an almost solid mass. The bacteria are thus surrounded by what may be termed a phagocytic wall which quite effectually prevents their spread, provided opsonins are present in sufficient quantities to enable the leucocytes to engulf and destroy the organisms. These leucocytes invade the diseased tissues and eventually destroy the bacteria present. This is not always easily accomplished, as many of the leucocytes themselves are destroyed by microörganisms. The pus found in such lesions is composed of a mixture of blood serum with white blood corpuscles or leucocytes, bacteria, and disintegrated tissue.

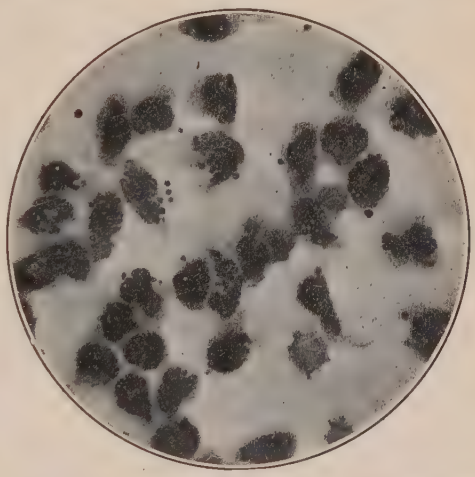


FIG. 151. — Stained mount of pus, showing the white blood cells with bacteria lying both within and without. (× 1000.)

It is only within the last half-century that pus production has been looked upon as an abnormal process. It was formerly supposed to be a part of the natural process of healing of wounds. Boils and abscesses were supposed to be efforts on the part of the body to rid itself of poisonous substances found in the blood. Something of this idea still persists, for one not infrequently hears the expression that a boil or series of boils is useful in that it tends to purify the blood. When it was determined, however, that microörganisms are in all cases the cause of suppuration, efforts

were made by the use of antiseptics, to prevent their development in wounds. This led to a great increase of efficiency in surgical operations. Another great step in advance was the introduction of what may be termed *aseptic surgery*, in which every effort is used to prevent the introduction of microörganisms into wounds by the use of sterile instruments, by careful disinfection of the skin, etc. It is practically impossible to prevent the introduction of all organisms, inasmuch as some are usually present in the deeper layers of the skin. The natural body immunity will ordinarily dispose of these, provided other organisms are not introduced in great numbers.

Staphylococcus aureus and Staphylococcus albus

Synonyms of Staphylococcus aureus. — *Micrococcus pyogenes aureus*, *Staphylococcus pyogenes aureus*, *Micrococcus aureus*.

Synonyms of Staphylococcus albus. — *Micrococcus pyogenes albus*, *Staphylococcus pyogenes albus*, *Micrococcus albus*.

The first definite report of the presence of microörganisms in pus is that given by Ogston in 1881. Rosenbach in 1884 grew them in pure culture on artificial media and differentiated the two species under consideration. These microörganisms are present quite commonly upon the skin and hair of man and animals. They are also commonly found in the mouth, and not infrequently in the intestines. They are not uncommon in dust.

Morphology and Culture. — These organisms are spherical, occasionally where they occur as diplococci they may be somewhat flattened at the point of contact. They are commonly designated staphylococci, as the cells ordinarily occur in irregular masses. The cells are somewhat less than 1 μ in diameter, usually between 0.7 μ and 0.9 μ. They are easily stained by the common laboratory aniline dyes and are gram positive.

They grow well upon the ordinary laboratory media. Pure cultures frequently may be obtained by smearing a small drop of pus over the surface of an agar slant by means of a platinum wire or loop. Often, however, they do not occur in pure cultures, and it is necessary to plate in order to get the separate distinct

colonies. Milk is usually curdled, becomes slightly acid, and eventually the curd is digested. Colonies upon gelatin plates or stab cultures in gelatin show rapid liquefaction. The principal point of difference between these organisms is that the *Staphylococcus aureus* upon most of the culture media produces a golden yellow pigment, while *Staph. albus* is white. These organisms are quite resistant to drying. This accounts for the presence of living organisms of these species in the air. They are easily destroyed by disinfectants; 60° C. for one-half hour is usually sufficient to destroy all cells; occasionally, however, strains are found that require heating to a higher temperature.

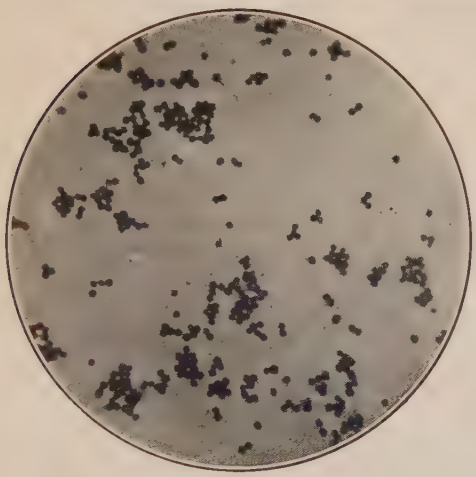


FIG. 152. — *Staphylococcus aureus.* (× 1000.)

Disease Production. — It is not probable that these organisms produce infections through their ability to form toxins, although small quantities of toxin have been demonstrated to be present. It has already been noted that these organisms are associated with a great variety of pyogenic infections, abscesses, carbuncles, boils, furuncles, and acne. Occasionally they may gain entrance to the blood stream and produce septicemia or pyemia. In some cases, also, the organisms are carried by the blood stream to the bone marrow and cause inflammation (osteomyelitis). Occasionally, too, they may settle down upon the lining membrane of the heart, or the heart valves, and cause one type of heart disease (endocarditis). Inflammation of the udder in cows is also occasionally found to be caused by organisms of this type. It sometimes happens, however, that an individual who is suffering from some chronic disease, such as tuberculosis, may have a secondary in-

fection produced by these organisms which may prove to be the immediate cause of death.

Immunity. — Inasmuch as toxins are not important in causing the changes in the body produced by these organisms, antitoxins are not of any use in curing the infections. Vaccines, particularly bacterins or killed cultures of these staphylococci, have been

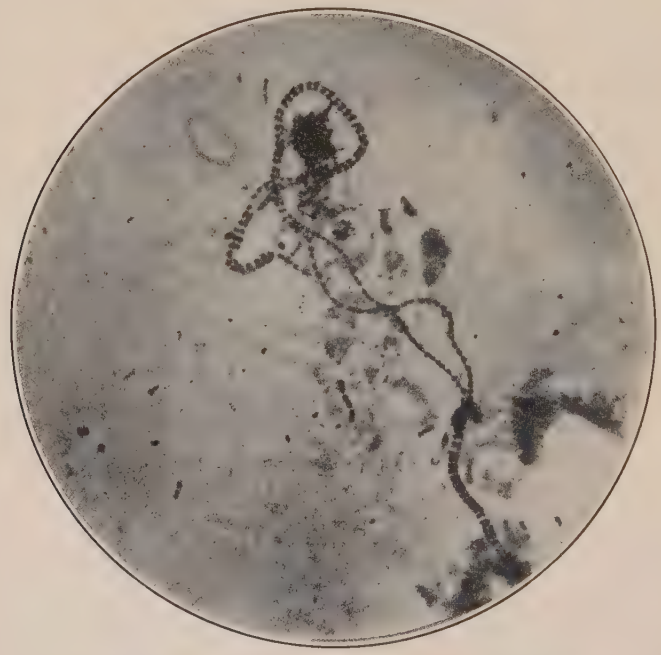


FIG. 153. — *Streptococcus pyogenes*, a single long chain from sputum. (× 1000.)

extensively tested in cases of chronic suppuration. These have been most successful where they are prepared from cultures taken from the particular individual to be treated. For example, cases of acne have been cured by obtaining pure cultures of the organism present in the pustules or pimples, growing these organisms upon artificial media, suspending them in physiological salt solution, and heating them to sufficient temperature to destroy them. These are then injected in small quantities subcutaneously. They cause

more or less reaction on the part of the tissues, but if properly used, seem to incite the body to an increased production of opsonins. These aid the leucocytes in ridding the body completely of the troublesome organisms. Such *autogenous vaccines* have been used also in cases of furunculosis.

Transmission. — Inasmuch as these organisms are always to be found upon the surface of the body, it is very difficult to prevent wound infection other than by the maintenance of a high degree of general body resistance. The various strains of these organisms isolated differ considerably in their virulence.

Streptococcus pyogenes

Synonyms. — *Streptococcus erysipelatis, Streptococcus puerperalis.*

This organism was isolated and described at about the same time as were the *Staphylococcus albus* and *Staph. aureus*. At first it was believed that distinction was to be drawn between the organisms isolated from erysipelas and those from other types of inflammation, but it was soon decided that these did not differ sufficiently to justify their separation into distinct species.

There seems to be little doubt but that several distinct species should be recognized, although the evidence of differentiation is not very good from the standpoint of morphological or cultural characters. It is convenient to separate the organisms of septic sore throat and scarlet fever from the true *Streptococcus pyogenes*. The differentiation is largely on pathogenic relationships.

Undoubtedly what is here included under the single specific designation of *Streptococcus pyogenes* really constitutes a group of species, an adequate basis for their differentiation is even yet not satisfactorily worked out. Some of the designations used with their significance may be noted. When streptococci are grown upon agar containing blood, some types produce a green coloration and are included in the name *Streptococcus viridans*, others produce hemolysis of the red cells about the colonies and are grouped as *Streptococcus hemolyticus*. Probably each of these names in turn comprise groups of species. Names have been given to several of those into which *Str. viridans* may be divided; among them *Streptococcus fecalis* for the form (sometimes termed the entero-

coccus) commonly present in human feces, the *Streptococcus equinus* from horse feces, the *Streptococcus buccalis* from the mouth, etc. These organisms vary from those which are highly pathogenic to those which are relatively non-virulent. Some of them are closely related to the *Streptococcus lactis* of milk.

For the purpose of this discussion it will suffice to consider the streptococci producing disease in man, or present upon the skin or in the body cavities as belonging to a single species. The organism does not grow as well upon artificial media nor adapt itself as readily to conditions in nature outside of the body as do the staphylococci. It is not uncommon upon the surface of the normal healthy skin, is quite constantly present in the mouth and throat, and may easily be isolated from the feces.

Morphology and Culture. — This organism is a coccus occurring in chains, sometimes short and consisting of three or four elements only, at other times very long, consisting of many cells. Individual cells are about 1.0 μ in diameter. This coccus is non-motile, does not produce spores, is easily stained, and is gram positive. It is easily isolated from the lesions which it produces and grows well upon most laboratory media, particularly when a sugar is present. The colonies developing on agar and gelatin plates are usually small, rarely larger than a pinhead. At first, they are transparent, later they become opaque. No pigment is formed and gelatin is not liquefied. When grown upon an agar slant, there is a tendency to form separate colonies rather than for the bacteria to grow together in a continuous mass, as generally occurs with the Staphylococci. Milk is coagulated, with the production of lactic acid. This last characteristic relates this organism to the *Streptococcus lactis* already described among the lactic acid bacteria. *Str. pyogenes* is aërobic and facultative anaërobic. It grows best at blood heat, but will also develop at room temperature and below. Sixty degrees C. for fifteen minutes is usually sufficient to destroy this organism, but sometimes strains are encountered which will resist higher temperatures.

Disease Production. — There is probably no species of organism which shows more marked variation in virulence than does *Str. pyogenes*. In some cases the cultures isolated may show absolutely

no power of disease production when injected into suitable laboratory animals such as the rabbit. In other cases, cultures may be so virulent that the introduction of a very few organisms is sufficient to produce fatal results. On the whole, the infections produced by this organism resemble those of the Staphylococci, but are usually more severe. It is found that the virulence can be increased by repeated inoculations of animals, and by reisolation from their bodies. Decrease in virulence occurs when the organism is grown for a considerable time upon artificial media. The exact mechanism of disease production is not thoroughly understood, but the organism does not produce toxins in sufficient quantities to account for the reaction in the body.

Streptococcus pyogenes is the cause of a considerable number of primary infections in man and is also important as a secondary invader. It is sometimes found in wounds, but is not as commonly present as are the Staphylococci. Occasionally an unusually virulent streptococcus may gain entrance to the blood stream and cause septicemia or blood poisoning. When growing in a tissue it is possible that it may penetrate a blood vessel wall with the resultant formation of an infected blood clot which may later break up, and when these particles are carried to other parts of the body, lesions may be developed there, producing pyemia. Erysipelas is a severe inflammation of the skin caused by the presence of large numbers of these organisms in the lymph spaces of the subcutaneous tissue. It seems to be due to a particular lack of resistance on the part of the individual and unusual virulence on the part of the microörganism. Inflammation of the tonsils (tonsillitis), of the intestines in children (enteritis), or other inflammations of the mucous membranes may be caused by *Str. pyogenes*. Puerperal fever, which is primarily an infection of the mucous surfaces after childbirth, usually followed by an infection of the adjacent tissues and even of the blood stream, is usually due to *Str. pyogenes*. It has been found that the organism producing erysipelas is particularly virulent in this respect. So-called heart trouble is not uncommonly caused by organisms gaining entrance to the blood stream and localizing upon the heart valves and adjacent membranes. Here they produce eventually more or less

distortion of the valve, leading to valvular insufficiency. Sometimes cauliflower-like growths occur upon the surface of these valves as a result of irritation by the organisms, and these may break off and be carried by the blood stream to various parts of the body, sometimes effectually stopping the flow of blood to some particular tissues. Invasion of the blood stream may also lead to localization of the organisms in the joints or in the bones, producing one of the many types of disease termed rheumatism (arthritis). It is probable that in a large proportion of these cases of endocarditis and arthritis, the organisms gain entrance to the blood stream primarily through the tonsils. Inflammation of the udders of cows is usually produced by this or very closely related organisms. Milk from such an udder, of course, is not fit for human consumption, as the organism when introduced in large numbers from such a source is usually of a highly virulent strain and may produce inflammation of the intestines and various other disturbances, particularly in children. This organism is likewise very important as a secondary invader in disease. Many of the relapses in typhoid fever, pneumonia, diphtheria, etc., are caused by this organism.

Immunity. — Bacterins may be prepared for the treatment and prevention of infection by these organisms in the same manner as has already been described for the staphylococci. Antistreptococcic sera have been prepared by repeated injections of broth cultures of the organism into the horse. Those organisms first injected are previously killed by heat. Later small injections of living organisms are made, and the injections are increased in size until a serum may eventually be prepared that when injected into the body will confer a considerable degree of immunity. It is unfortunately true that the antistreptococcic serum prepared for one strain of *Str. pyogenes* is not always efficient in protecting against another strain. This serum does not owe its curative effect to the presence of antitoxins. Probably opsonins are of importance, possibly also bacteriolysins.

Transmission. — Inasmuch as this organism is so common upon the surface of the body and alimentary tract, it is quite impossible to free the body from it. The strains that are ordinarily found under these conditions, however, are not the most virulent. Some-

times it is necessary that precautions be used to prevent the transmission of virulent strains from one individual to another. The intimate relationship, for example, existing between erysipelas and puerperal fever has already been noted.

Streptococcus scarlatinæ

There seems to be a good reason to believe that a streptococcus is the causal organism in the disease scarlet fever. The fact that streptococci are usually found in the throats of those suffering from scarlet fever has long been known, but proof of their causal relationship has not been clear. More recently it has become probable that these organisms represent a distinct group of hemolytic streptococci, and may therefore be regarded as a distinct species, *Str. scarlatinæ*. The most convincing evidence of the etiologic significance of this organism has come through the demonstration of G. F. and Gladys Dick that filtrates of broth cultures of this organism when injected intracutaneously give a decided local reaction in a considerable proportion of individuals, but not in those who have been immunized by an attack of the disease. This so-called Dick test is used extensively as a means of recognizing non-immune individuals.

There seems to be good evidence that the scarlet fever streptococcus produces a specific toxin, and that this is in part at least responsible for the pathogenesis of the organism. Apparently an antitoxin is produced in the blood of convalescents.

It has also been proved practicable to produce a curative antitoxic serum by the immunization of the horse. This has proved reasonably effective in the prevention and cure of scarlet fever.

CHAPTER XXXVII

GROUP OF SPECIFIC COCCI

A NUMBER of specific diseases of man and animals are produced by cocci. The most important of these organisms are *Diplococcus pneumoniæ*, a cause of pneumonia, *Neisseria meningitidis*, the cause of epidemic cerebrospinal meningitis, or spotted fever, and *Neisseria gonorrhœæ*, producing gonorrhea.

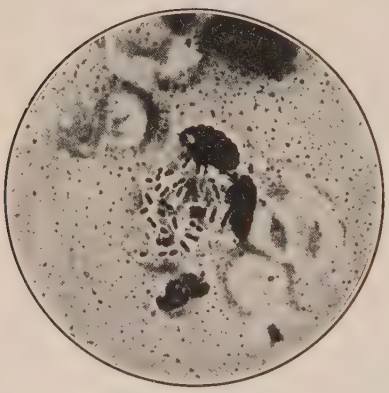


FIG. 154. — *Diplococcus pneumoniæ* in sputum. (After Günther.)

These organisms are grouped together inasmuch as they are all cocci and all produce specific diseases. They differ, however, in many other respects, for some are gram negative (members of genus Neisseria), others gram positive (members of genus Diplococcus), some grow readily on artificial media, and some do not.

Diplococcus pneumoniæ

Synonyms. — Pneumococcus, *Diplococcus pneumoniæ*, *Diplococcus lanceolatus*, *Streptococcus pneumoniæ*, *Micrococcus pneumoniæ*, *Micrococcus lanceolatus*.

This microörganism is the common cause of infectious pneumonia. It was first definitely associated with pneumonia 1885 by Fränkel.

Morphology and Culture. — The organisms usually occur in pairs, rather more rarely in chains of four to six or more individuals. The cells are spherical when isolated, but when in pairs they are generally flattened at the point of contact, with the opposite sides somewhat elongated and pointed. The organism may be readily recognized in stained mounts from the sputum, where it is found to have a capsule. It stains readily with the common aniline dyes and is gram positive. Under some conditions in certain culture media it is sometimes difficult to differentiate this organism from the *Streptococcus pyogenes*.

A character most commonly employed for differentiation of streptococcus and pneumococcus is the fact that the latter is readily soluble in bile, while the former is not. In most cases pneumococci are able to ferment inulin with the production of acid, while streptococci do not.

The growth of this organism is never luxuriant upon media, though it will develop on most of them with the exception of the potato. Minute dewdrop-like colonies form upon the surface of the medium, usually not coalescing. Acid is produced in milk and this medium is coagulated. Growth is better in a mixture to which glycerol and blood serum have been added. The organism grows best at blood heat, and will develop little or not at all at room temperatures. It is usually destroyed by direct exposure to sunlight, and is killed by drying, although when dried in sputum it may be somewhat more resistant.

Disease Production. — The pneumococcus seems to be present normally in the mouths of a considerable percentage of individuals. If sputum from such an individual is injected into a guinea pig, or better intravenously into a rabbit, the animal will frequently die of an acute septicemia, and the organism can be demonstrated without difficulty in the blood. Cultures from various individuals and from various sources show great differences in their virulence or power to produce disease. Pneumonia is one of those diseases which usually requires a considerable diminution in the natural resistance of the individual, and possibly the presence of an organism of unusual virulence.

The work of Dochez and Gillespie has shown that there may be differentiated several distinct types of the pneumococcus, the

differentiation being based primarily on the immunological reactions, particularly agglutination. These were termed Types I, II, III, and IV. The first three are relatively distinct, the fourth is a group which contains those organisms which do not belong in one of the other types. Group IV does not in general produce the more serious cases of pneumonia.

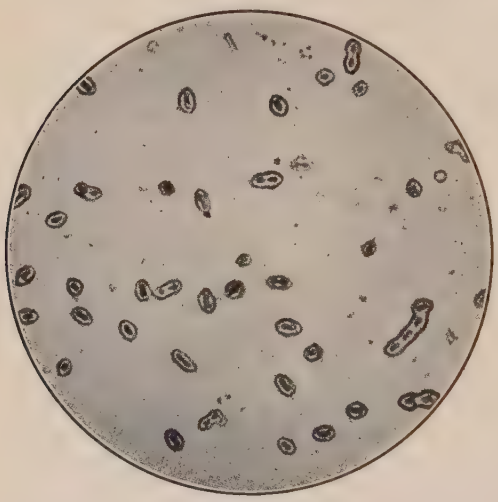


FIG. 155. — *Diplococcus pneumoniæ*, stained preparation showing capsules. (Buerger in *Journal of Infectious Diseases*.)

The tissues of the portion of the lung infected by the pneumococcus become congested with blood, and blood plasma passes out into the air sacs (alveoli). Here the fibrinogen coagulates (is converted into fibrin) and that portion of the lung becomes *hepatized*, or liverlike, in consistency. Sometimes red blood corpuscles likewise find their way into these alveoli, and a section through the lung tissue shows it to be red in consequence. The leucocytes later invade the tissues and air sacs, and they become gray. The microörganisms multiply in the tissues and alveoli, and in some cases gain entrance to the blood stream. In the latter event they may produce septicemia. The damage done to the lung tissue itself does not seem to be sufficient to account for the grave disturbances in general health characteristic of this disease. It is possible that poisons, probably endotoxins, are produced in sufficient quantities by this organism to account for the fever. During convalescence the fibrin and other material in the air sacs undergo autolysis as a result of digestion by enzymes secreted by leucocytes and possibly also by the lung cells.

Immunity. — No antitoxin has been prepared for this disease inasmuch as a toxin has never been demonstrated for it. It is probable that recovery from the disease is largely due to the production of opsonins in the blood and tissues as a result of the presence of the organism. Immunity does not last long in pneumonia. As a matter of fact, complete recovery is sometimes followed by an increased susceptibility to the disease. Antipneumonic serum has been prepared by the systematic injection of killed and living cultures of the pneumococcus into animals, but its use has not proved to be entirely satisfactory. This is probably due, in part at least, to the fact that an antiserum prepared by use of one type of pneumococcus is not effective against infections produced by other types. In fact, the antiserum specific for Type I pneumococcus is the only one which has proved particularly effective in man.

Transmission. — Pneumonia is not usually regarded as a contagious disease, although it is undoubtedly true that when a number of individuals having a low grade of resistance are associated together, the disease sometimes assumes epidemic form. It is probable that in most cases the organisms responsible for the disease are already present in the mouth and throat, and begin to develop as a result of a diminution of natural body resistance.

Neisseria meningitidis

Synonyms. — *Diplococcus intracellularis meningitidis, Micrococcus weichselbaumii; Streptococcus meningitidis,* meningococcus.

The organism is the specific cause of the disease known as epidemic cerebrospinal meningitis in man, sometimes also called spotted fever. The organism was first observed by Weichselbaum in 1887. The meninges are the membranes which cover the brain and spinal cord; cerebrospinal meningitis, therefore, is the name applied to an acute inflammation of these membranes.

Morphology and Culture. — Material for a mount of the organism as it occurs in the exudate from the meninges may be obtained by thrusting a hypodermic needle between two of the lumbar vertebræ. The fluid is under sufficient pressure so that a few drops at least will pass out. These can be collected upon glass slides or

used to make cultures. In the stained mounts the organisms are found within the white blood corpuscles or phagocytes. They usually occur as diplococci or in groups of fours. When grown on culture media, the cells are about 1 μ in diameter, usually in pairs, sometimes in chains. The organism is easily stained, but is gram negative. This fact differentiates it sharply from the pneumococcus previously described.

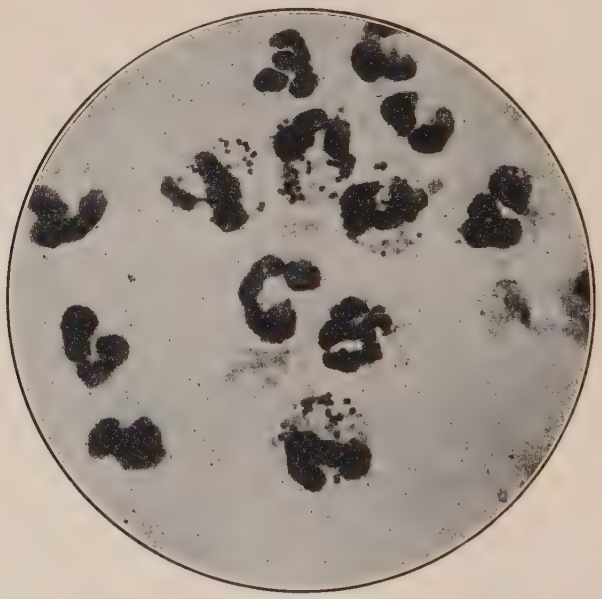


FIG. 156. — *Neisseria meningitidis*, in a preparation of pus from a brain abscess. (Flexner.)

The meningococcus, when first isolated, does not grow well upon culture media. It is customary, therefore, to use a medium containing blood serum. Upon this medium a white viscid coherent colony is developed. It does not liquefy gelatin, and milk is not changed. It produces acid from dextrose and maltose, but not from sucrose or levulose. Ability to ferment maltose is one character differentiating this organism from the gonococcus. It does not retain its vitality for long periods upon artificial media. Frequent transfers are therefore necessary to maintain the cultures

in vigorous condition. It is destroyed very quickly by desiccation and by the action of disinfectants.

Disease Production. — This organism does not readily produce disease in laboratory animals unless injected in large quantities. However, the injection of pure cultures into some animals, such as the monkey, has caused a reproduction of the disease practically as it develops in man. The organism grows upon the surface of the meninges, causing an acute inflammation with a purulent exudate. The manner in which the organism gets into the brain and spinal cavities is not well understood. It has been found on the nasal mucous membranes of those having the disease and of those associated with such individuals. It is probable that the organism gains entrance in some manner to the blood stream, and finds favorable conditions for its development on the meninges; possibly it enters through lymph channels. The disease is highly fatal. Until the introduction of the serum noted below, few cases recovered.

Immunity. — An immune serum has been obtained by the repeated injection of the horse with pure cultures of this organism. The blood serum is used. Several cubic centimeters of the fluid surrounding the meninges of the patient are removed by means of the hypodermic needle as explained above. About the same amount of the immune serum is injected to take its place. The fact that the immune serum comes into immediate contact with the organisms on the meninges seems to give optimum conditions for its action, and very favorable results have been reported as a consequence of its use. It is not certainly known to what the antiserum owes its immunizing power, probably to its content of both bacteriolysins and opsonins.

The problem of immunization against meningitis is complicated (as in pneumonia) by the existence of several distinct types of meningococci. Antiserum effective against one type may be quite ineffective against another. At least four types have been differentiated. The difficulties may to some degree be obviated by the use of a polyvalent serum, that is, one which has been prepared by immunizing a horse (usually) against several types simultaneously.

Transmission. — It is probable that the disease is transmitted from one individual to another by more or less intimate contact,

the use of common drinking cups, mouth spray, etc. The disease sometimes appears in epidemic form and is usually confined to children, although adults are not entirely immune.

The disease is rather difficult to combat because of carriers. The organism apparently first invaded the naso-pharynx, where it finds conditions suitable for growth. In a considerable percentage of these the organisms never invade the meninges, but remain localized at the initial site of infection. Such individuals are carriers, and their recognition is a matter of considerable importance in controlling an epidemic of the disease. Carriers are recognized by isolation of the characteristic organisms from the throat.

Neisseria gonorrhœæ

Synonyms. — *Diplococcus gonorrhœæ*, *Micrococcus gonorrhœæ*, Gonococcus.

The gonococcus is the specific cause of gonorrhea and its related disorders in man. Gonorrhea is primarily a disease of the mucous membranes of the urogenital tract. In some cases the membranes of the eye may be infected. It is one of the most widespread and serious of human diseases. The organism was first observed in gonorrheal pus by Neisser in 1879, but it was not cultivated until 1885 by Bumm.

Morphology and Culture. — The gonococci in stained mounts of gonorrheal pus are usually to be found within the white blood cells. Typically they occur in pairs, flattened on the proximal ends and rounded on the opposite. They may be described as coffee bean shaped. The cells when free are spherical. They are about $1.6 \times 0.8\,\mu$. Chains are rarely formed in culture media. They stain readily with the aniline dyes and are gram negative. This point is of considerable importance in the diagnosis of infections, as most other pus-producing cocci are gram positive. The organism is cultivated with difficulty when first isolated in pure cultures, even upon a serum medium. After cultivation and a succession of transfers, growth becomes more luxuriant. The growth consists of minute, discrete, transparent colonies.

The gonococcus is aërobic, easily destroyed by drying, and even

in culture media frequent transfers are necessary to keep it alive. It grows only at temperatures within a few degrees of blood heat. It is readily destroyed by disinfectants and by heat. The possibility of transference of the infection other than by direct contact is rather remote, owing to the readiness with which the organism succumbs to unfavorable environment.

Disease Production. — Gonorrhea is a disease characteristic of man alone. It has not generally proved possible to transmit the

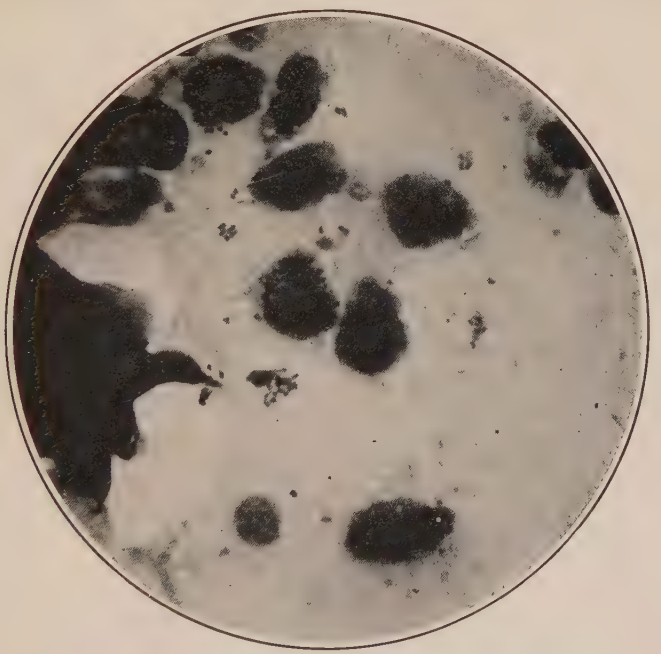


FIG. 157. — *Neisseria gonorrhœæ* in gonorrheal pus. (× 1000.)

typical disease to animals, although local suppuration and necrosis and even death may be occasioned by injections of the organisms. Man has been infected by the use of pure cultures, so that there is no question as to the causal relationship of the organism to the disease.

In the male the primary infection is manifested by an inflammation of the urethra. Quite characteristic is the production of a

thick, creamy pus. The inflammation may extend to the prostate gland, the bladder, the seminal vesicles, and even to the epididymis. After a time the secretion changes in character, and may cease. The disease is apt to persist in chronic form. Long-continued inflammation may lead to the formation of scar tissue, and constriction of the urethra, or stricture. The organism sometimes spreads to other parts of the body, particularly the joints, with consequent production of gonorrheal rheumatism; more rarely it may cause endocarditis. The disease may persist in latent form for a considerable period of time, even years, after the initial infection, and may recur in acute form.

In the female the initial infection is usually of the vulva. It may spread to the urethra and bladder, more commonly to the uterus. A not infrequent sequel is involvement of the Fallopian tubes, requiring surgical intervention and consequent sterilization. Blindness resulting from inflammation of the eyes and adjacent membranes of the newborn is usually the result of gonorrheal infection of the mother. When there is reason to suspect such infection, it is customary to instill antiseptic solutions, usually silver compounds, into the conjunctiva of the child to prevent the development of the organism.

Particularly to be emphasized is the long period of time during which an infected individual may be able to transmit the infection, even though there has apparently been complete recovery.

Immunity. — Very little if any immunity is conferred upon an individual by an attack of gonorrhea. Satisfactory methods of passive immunization have not been developed. Vaccines consisting of suspensions of gonococci killed by heat have proved useful, particularly in chronic infections. The disease, after it has passed the acute stage, is difficult to cure by the local use of disinfectants.

Transmission. — The disease is transmitted in the great majority of cases by contact. Epidemics have been observed, however, in children's hospitals due to the common use of towels, washbasins, etc. There is a possibility of such transmission in public lavatories and toilets, although infection in this manner is undoubtedly rare.

CHAPTER XXXVIII

DIPHTHERIA GROUP

The organisms belonging to this group are gram positive bacilli which when properly stained show characteristic metachromatic granules. They are non-motile and do not produce spores. The most important organism belonging to the group is *Corynebacterium diphtheriæ*. Some organisms related to *Cor. diphtheriæ* have been described under the name *Cor. pseudodiphtheriæ*. They differ in some degree morphologically from the true diphtheria organism and are not capable of producing disease. The term diphtheria as originally used by physicians indicated a pathologic condition in which there was more or less extensive death of the cells of the mucous surfaces and formation of false membranes of fibrin usually in the throat or nose. This type of inflammation was said to be *diphtheritic*. It has been found to be most commonly caused by *Cor. diphtheriæ*, but other organisms have been described which can bring about somewhat similar changes. The term diphtheria as now commonly used, however, indicates simply a diseased condition in which the specific organism, *Cor. diphtheriæ*, is present, even though the typical false membrane is not produced.

Corynebacterium diphtheriæ

Synonyms. — *Bacillus diphtheriæ, Bacterium diphtheriæ, Mycobacterium diphtheriæ.*

This organism is the cause of the disease diphtheria in man. The disease is not to be confused with somewhat similar diseases in animals sometimes termed diphtheria. These are caused by organisms which are totally distinct. *Corynebacterium diphtheriæ* was first described by Klebs in 1883, and Loeffler in 1884 studied it in pure culture. Roux and Yersin between 1888 and 1890 dem-

onstrated that it produces a characteristic toxin and that many of the symptoms and lesions of diphtheria can be duplicated in laboratory animals by the injection of bouillon filtrates.

Morphology and Culture. — When grown on culture media, the diphtheria bacillus shows such great variations in its morphology that some writers believe it to be more closely related to fungi than to the true bacteria. For example, club-shaped and branching forms are not uncommon. Typical organisms when stained with methylene blue are seen to be rods varying from 0.4 to 1.4 μ in diameter and from 1.5 to 3.5 μ or even more in length. Sometimes they stain uniformly, but more commonly show deeply stained bands or granules in the cell which give to the cell contents a granular or barred appearance. The organism does not produce spores or capsules, is nonmotile and gram positive. Cultures may be secured upon blood serum slants directly from the throat of a patient with diphtheria. The diphtheria organism kept at blood heat upon this medium grows at first more rapidly than most of the other bacteria that may be present. It forms upon the surface of this medium, sometimes within twelve to eighteen hours, small pin point like, translucent dots which later become opaque, gray colonies. It does not grow very readily, at first at least, upon agar and gelatin unless glycerol be added to them. After cultivation for some time on artificial media, development upon agar or gelatin is somewhat more luxuriant. It produces no observable change in milk.

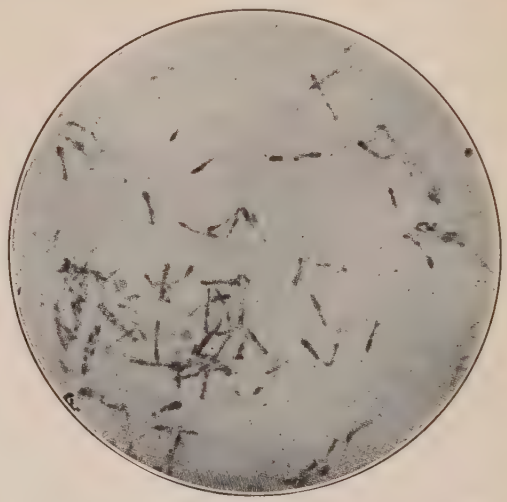


FIG. 158. — *Corynebacterium diphtheriæ*. (After Epstein in *Journal of Infectious Diseases*.)

In bouillon after a time a delicate film will form, and transfer of this film to new tubes of broth will confine the growth practically to film formation. This, it will be remembered, is the method employed in the manufacture of diphtheria toxin.

Corynebacterium diphtheriæ is aërobic. It retains its vitality for a long time when grown upon culture media. It is not particularly resistant to drying, but when in sputum or diphtheritic membrane it may be dried for considerable periods, in some cases, months, without being killed. The temperature of pasteurization of milk is sufficient to destroy the organism. Gelatin is not liquefied.

Disease Production. — Diphtheria is typically a disease of man, particularly of children. It is a true toxemia, that is, the organisms are confined to a relatively small area of the mucous membrane of the nose or throat, and growing there, produce their characteristic toxin, which is absorbed by the blood and injures various internal organs, such as the liver and the heart muscles. The false membrane as it occurs in the throat results from the direct injury to the mucous membranes by the organisms and their toxin, causing death of the cells, and a considerable degree of inflammation and swelling consequent upon the inflammation of the tissues. The blood vessels are engorged with blood, and blood plasma is thrown off. The fibrinogen is converted rapidly into fibrin, and when mixed with desquamated cells of the mucous membrane and red and white blood corpuscles, constitutes the false membrane. In this membrane conditions are usually quite favorable for the continued growth of the organism. It sometimes happens that the membrane becomes large enough to cause death by asphyxiation due to the occlusion of the air passages. The pharynx is most commonly affected, the larynx and the nasal mucous membranes may also be involved. Occasionally the organism may invade the middle ear through the Eustachian tube. Sometimes the diphtheria bacillus may develop in wounds.

Immunity. — A person who has had diphtheria is thereafter relatively immune to the disease, at least for a time. This is undoubtedly due to the development in the body of antitoxins which neutralize the poison. According to Ehrlich the diphtheria bacillus produces two kinds of poisons, differentiated as toxin and

toxone. The former gives rise to the acute symptoms of poisoning which one observes during the height of the disease, the latter is believed to cause the paralysis that may occur during convalescence. The disease may be prevented by the injection of antitoxin and the same means is generally employed for cure. The method of manufacturing diphtheria antitoxin has already been considered under the heading of immunity (Chapter XXXIII). The use of antitoxin has much diminished the death rate from diphtheria. Vaccination by mean of mixtures of toxin and antitoxin are extensively utilized.

Diagnosis. — Most states and cities maintain bacteriological laboratories to assist physicians in diagnosing such diseases as diphtheria. The method consists in passing a sterile swab over the surface of the inflamed area and drawing this over the surface of some suitable culture medium, usually Loeffler's blood serum. The culture so obtained is then sent or taken to a laboratory and incubated from twelve to eighteen hours. Mounts stained with Loeffler's methylene blue show the characteristic organisms with metachromatic granules. It is sometimes possible to make diagnoses of diphtheria from stained mounts of material taken directly from the throat.

The *Schick test* is frequently used in an effort to determine susceptibility to diphtheria. The test consists in the injection of a minute dose of diphtheria toxin into the skin. Susceptibility to diphtheria is shown by reddening and swelling of the tissues.

Transmission. — The most common method of transmission of diphtheria is by the use of common drinking vessels, and by putting infected articles such as lead pencils in the mouth. It may sometimes be contracted by the inhalation of infective droplets. Several epidemics have been shown to be due to milk infection. The latter, of course, arises from the fact that the organism gains entrance to the milk from some individual who handles it, and not directly from the cow.

CHAPTER XXXIX

PLAGUE GROUP

The organisms belonging to the plague or hemorrhagic septicemia group of bacteria are all characterized by being short, oval rods, non-motile, non-spore producing, and gram negative. When grown in the body (sometimes also in culture media) they show polar granules when stained by appropriate dyes. To this group of organisms the generic name Pasteurella is usually applied. Some authors include them all in the genus Bacterium. The organisms causing several diseases of animals are included in this group. Among these are hemorrhagic septicemia of cattle, swine, rabbits, and fowls. The last named (fowl cholera) has been most studied. The causal organism is *Pasteurella choleræ-gallinarum.* Two species are known to produce disease in man, the *Pasteurella pestis,* the cause of plague, and *Pasteurella tularensis,* the cause of tularemia.

Pasteurella pestis

Synonyms. — *Bacillus pestis, Bacillus pestis bubonicæ. Bacterium pestis.*

This organism was described independently by Yersin and Kitasato in 1894 as the cause of bubonic plague. The disease is one which is endemic in parts of China, and possibly in India as well. History shows that at several different times an epidemic of this disease has spread over the entire civilized world. Such,

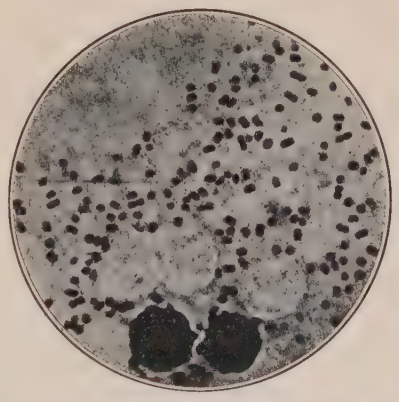


Fig. 159. — *Pasteurella pestis.* (After Günther.)

for example, was the black death which devastated Europe. In the past several decades India and portions of China have been in the midst of such an epidemic, and in consequence the disease has been transported to practically every port of prominence in the various countries. It has not succeeded generally, however, in obtaining a secure foothold in these countries.

Morphology and Culture. — *Pasteurella pestis* is a small bacillus 0.5–0.75 $\mu \times$ 1.5–2 μ. It is usually single, rarely occurring in chains. In certain culture media involution forms are commonly observed. When smears are made directly from infected tissue, the organism exhibits a very decided bipolar staining. It may be easily isolated from infected lymph glands in pure culture. Occasionally it may be obtained from blood. It grows readily upon various culture media, but poorly or not at all upon potato. It grows either with or without oxygen. It has an optimum temperature between 25° and 30° C. It is not particularly resistant to heat, drying, light, or disinfectants.

Disease Production. — Bubonic plague has come to be regarded as a disease characteristic of certain rodents, particularly the rat and certain burrowing forms. It is readily transmitted to man. Mice, rats, guinea pigs, rabbits and other rodents may be readily infected.

The disease in man manifests itself usually in one of three types depending apparently upon the method of infection and virulence of the organism. Usually the organism enters through the skin, and causes the production of buboes, that is, large swellings of lymph glands, these frequently ulcerating and discharging pus superficially. This is the characteristic of bubonic plague. This stage is frequently followed by invasion of the blood stream and consequent septicemia. In some cases the organism is sufficiently virulent to invade the blood stream almost immediately, with the production of the septicemic type of plague. Usually in this disease there are hemorrhages in the subcutaneous tissues, with consequent formation of darkened areas of skin. This is characteristic of the hemorrhagic septicemias. It was this darkening of the skin which gave the name " black death " to the disease. The third type of the disease, the penumonic, is probably

usually contracted by the inhalation of infectious droplets. It is an acute and quickly fatal type of pneumonia.

Immunity. — Those who recover from an attack of plague show a relatively high degree of immunity. The organism does not produce a toxin. Antisera have been prepared, but the results obtained have not always been favorable. The antiserum is prepared by repeated injections into suitable animals, such as the horse, of killed cultures of this organism followed by increasing doses of living bacteria. Blood serum from such an animal which has developed a high degree of immunity is believed to confer a considerable degree of passive immunity upon the individual into whom it is injected. Active immunization by the injection of killed or even living attenuated cultures of bacteria has been extensively practiced, and has proved successful.

Transmission. — It is probable that the disease may be transmitted by the inhalation of infectious droplets, or by close contact with infected individuals. The more common method of transmission, however, is by the bite of fleas or through their excretions scratched into the skin. It has long been known that an outbreak of bubonic plague is frequently preceded by the appearance of the disease among rats and other rodents. It is now known that the rat fleas will leave the body of a rat which has died and may attack man. Bubonic plague is evidently a vermin disease. It may infect rodents other than the rat. It was found as a result of the investigations of an outbreak of the disease in San Francisco in recent years that some of the ground squirrels in the neighborhood of the city were diseased, and cases of direct infection of man from these animals have been reported.

Pasteurella tularensis

Synonym. — *Bacterium tularense.*

A disease somewhat resembling plague was discovered by McCoy and Chapin in 1912 in a California rodent, a ground squirrel. Later it was found that the disease is rather widespread among rabbits, and perhaps other rodents, almost throughout the United States. It has also been reported from Japan. It was found to be the cause of so-called deerfly fever in the western part of the

United States, apparently transmitted to man by flies that had previously bitten infected jack-rabbits. Later numerous cases came to light in which the infection came from handling rabbits which had been shot. The disease has been named tularemia after the organism, and this in turn after Tulare County in California, where it was first discovered.

Morphology and Culture. — *Pasteurella tularensis* is a minute gram-negative rod which in laboratory media exhibits a marked degree of plemorphism. It is grown with some difficulty in media. Those which have proved most satisfactory are coagulated egg-yolk and a blood glucose cystin agar. Acid (no gas) is produced from dextrose, levulose, mannose, and glycerol.

Disease Production. — Tularemia usually manifests itself by the development of ulcerated lymph nodes, usually those near the site of inoculation. In this respect it resembles somewhat the bubonic plague. In some cases the disease is somewhat typhoid-like in its manifestations. It is not usually fatal. A relatively permanent immunity is acquired as a result of recovery.

CHAPTER XL

INTESTINAL OR COLON-TYPHOID GROUP

The organisms of the intestinal group, as the name indicates, are characteristic members of the intestinal flora in health or disease. Some are also found living saprophytically in soil and water. They are all plump, gram negative rods, and do not produce spores. All are aërobic and some are facultative anaërobic. Some species are motile, others non-motile. This group is of importance because of several disease-producing bacteria that it contains, also because of the fact that the intestinal bacteria are the ones for which search is made in an examination to determine the potability of water.

The naming of the organisms of this group is in a somewhat chaotic condition. In many cases all of the organisms are included together in the genus Bacterium, in other cases the group is split up into a considerable number of genera, including Aërobacter, Escherichia, Proteus, Salmonella, Eberthella, Flexnerella, and possibly Alcaligenes. These all contain species that are of importance.

The group is divided into three subgroups, the basis for the classification being the ability of the organisms to ferment various sugars. The following chart, which gives the production of acid and gas from dextrose and lactose, will indicate the characteristics of the three subgroups.

Subgroup I Colon Aërogenes Subgroup			Subgroup II Intermediate Subgroup			Subgroup III Typhoid Dysentery Subgroup		
	Acid	Gas		Acid	Gas		Acid	Gas
Dextrose .	+	+	Dextrose .	+	+	Dextrose .	±	−
Lactose . .	+	+	Lactose . .	−	−	Lactose . .	−	−

The organisms of subgroup I, as will be noted, ferment both dextrose and lactose with formation of both acid and gas; those of subgroup II produce both acid and gas from dextrose but neither from lactose, and those of subgroup III may or may not form acid from dextrose but not from lactose, and gas is produced from neither of the sugars.

Within the confines of the subgroups the various species described are sometimes difficult to differentiate. One of the most satisfactory means that has been employed is the use of still other sugars and similar compounds to determine the fermentative powers of the related organisms. Some species can be differentiated from each other satisfactorily only by the use of specific agglutination reactions. In some cases the composition of the gas, that is, the ratio of carbon dioxide to hydrogen, is of assistance.

Subgroup I. Colon Aërogenes Subgroup

This subgroup includes a large number of species, for the most part non-pathogenic, many of them inhabitants of the intestines of man and animals, others may also develop saprophytically. Those which are parasitic primarily are of great interest inasmuch as their presence or absence in water is one of the best evidences of sewage contamination. Those which are non-parasitic are of significance because they may be confused with the preceding. All produce both acid and gas in the fermentation of both dextrose and lactose.

The organisms of this subgroup are frequently divided into genera, Escherichia and Aërobacter. The two genera may be differentiated by reference to the following diagnoses: —

Escherichia. — Gas produced from dextrose has the carbon-dioxide-hydrogen ratio 1–1. Acetoin (acetyl-methyl-carbinol) is not produced from dextrose, hence the Voges Proskauer test is negative. When grown in a special dextrose medium the hydrogen ion concentration is increased to a point such that the methyl-red indicator shows its acid color red, *i.e.*, the organisms are said to be methyl red positive. In general the bacteria of this genus do not utilize citric acid as a source of carbon.

Aërobacter. — Gas produced from dextrose has a ratio carbon dioxide-hydrogen 2–1. Acetoin is produced, *i.e.*, the organisms are Voges-Proskauer positive. They are methyl red negative, and utilize citric acid.

Occasionally organisms are encountered which are intermediate in one or more characters, but usually cultures of organisms of this subgroup can be allocated definitely to one genus or the other.

Each genus contains numerous species which may be differentiated from each other on the basis of fermentative and other reactions. The type of each genus (*Escherichia coli* and *Aërobacter aërogenes*) only will be discussed.

Escherichia coli

Alternative Name. — *Bacterium coli.*

Synonyms. — *Bacillus coli communis*, *Bacterium coli commune*, colon bacillus.

Escherich in 1886 isolated the organism from normal feces and called it *Bacterium coli commune.* The organism is quite commonly present in the intestinal tract in man and most animals. Whether or not it ever occurs in nature wholly independent of fecal contamination is an unsettled question. Undoubtedly it may continue to grow for some time outside of the body under favorable conditions, but it does not seem to be common, at least not on virgin soil and unpolluted water. Recent work seems to indicate quite clearly that the older reports of finding this organism in nature under conditions which made improb-

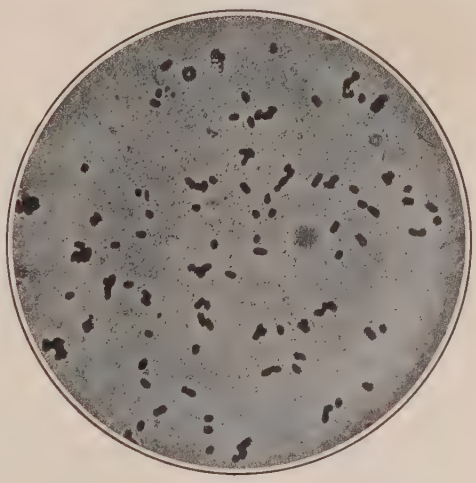


Fig. 160. — *Escherichia coli.* (× 1000.)

able fecal contamination were based upon confusion of this organism with *Bact. aërogenes*. Its presence in water is universally regarded as indicative of sewage pollution. Such water is not regarded as potable. This is not because *Escherichia coli* possesses of itself any marked pathogenic characters, for it is found in greater numbers in the normal human intestines than in grossly polluted water, but because its presence indicates sewage pollution and the probable presence of disease-producing bacteria such as the typhoid bacillus.

Morphology and Culture. — *Escherichia coli* is a plump rod 0.4–0.7 μ × 2.0–4.0 μ. Occasionally it is shorter, and when growing rapidly may resemble a coccus. It is usually single, but may form short chains. Neither spores nor capsules are ever produced. It is motile in young cultures, although never actively so. It stains readily and is gram negative.

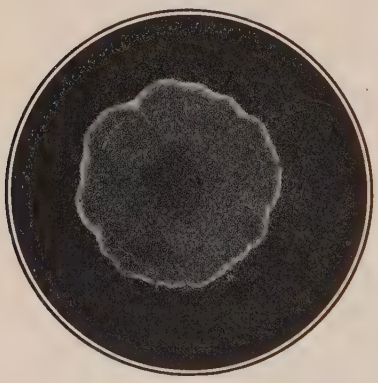


FIG. 161. — Colony of *Escherichia coli* on agar. (× 20.)

Upon gelatin plates the colonies are moist, grayish white, opaque, becoming darker and coarsely granular. Gelatin is not liquefied. In gelatin stabs a uniform filiform growth occurs along the line of puncture, and the growth spreads over the surface to some extent. Bouillon is clouded, sometimes with the formation of a membrane. A moist spreading growth appears upon the potato and the medium is darkened, sometimes becoming almost black. Milk is coagulated with the formation of lactic acid and gas. The curd shrinks, but is not digested, as this organism is not actively proteolytic.

Escherichia coli is an aërobe and in the presence of carbohydrates a facultative anaërobe. It grows best at blood heat, but develops well at room temperatures and even below. It is destroyed when heated to a temperature of 60° C. for 15 minutes. A considerable number of carbohydrates are fermented, among them dextrose,

lactose, and maltose. Other closely related strains ferment sucrose.

Indol is formed in Dunham's solution. This organism is not usually regarded as pathogenic, although many authors have ascribed disease-producing powers to it. This is largely due to the fact that they have not discriminated carefully between *Escherichia coli* and some of the other organisms belonging to the intestinal group. However, when broth cultures of this organism are injected intraperitoneally into a guinea pig, the animal dies, usually within three days. In man it has been known occasionally to invade the gall bladder. It has also been described as producing inflammation of the urinary bladder.

Methods used in the isolation and identification of this organism in water analyses will be discussed in Chapter XLVIII.

Aërobacter aërogenes

Alternative Name. — *Bacterium aërogenes.*

Synonym. — *Bacterium lactis aërogenes, Bacillus lactis aërogenes.*

This organism was first described by Escherich in 1885 as the cause of the souring of milk. Since that time it has been found repeatedly under the same conditions as *Escherichia* (*Bacterium*) *coli*, that is, it has proved to be one of the common intestinal organisms. There has been much confusion in the past relative to the importance of this organism because it has so frequently been confused with *Bact. coli.* Recent work seems to show that this organism, unlike *Bact. coli*, is not uncommon in nature outside the alimentary tract. It has been found repeatedly in soils and on grains, likewise it is abundant in water of streams during floods and high water. Its relationship to the souring of milk has already been discussed.

Morphology and Culture. — The *Aërobacter* (*Bacterium*) *aërogenes* differs from *Bact. coli* principally in the total absence of flagella, *i.e.*, the organism is non-motile. Under suitable conditions it is able to produce capsules when grown in milk. The colonies upon agar and gelatin are thicker, larger, and more slimy than those of the colon bacillus, and milk is curdled even more rapidly. This organism ferments dextrose, lactose, sucrose, and

starch with the production of both acid and gas. Cultures upon potato generally show gas bubbles. Indol is formed in Dunham's solution.

Disease Production. — This organism is not known to be pathogenic. In water analyses it is frequently confused with *Bact. coli*, inasmuch as they are so closely related. The differentiation from *Bact. coli* will be discussed later. It does not apparently have the same sanitary significance.

Subgroup II. Intermediate or Enteritidis Subgroup

Here are included those organisms which belong to the intestinal group and which ferment dextrose but not lactose with production of acid and gas. Two genera may be differentiated, Proteus and Salmonella. They may be distinguished as follows: —

Proteus. — Acid and gas produced from sucrose, gelatin usually liquefied. The most common species, *Proteus vulgaris*, has been reported as occasionally pathogenic. It is not of sufficient importance to be discussed further here. Its relationship to decay has been previously considered.

Salmonella. — Gas not produced from sucrose, gelatin not liquefied. A considerable number of species are included here, many of them pathogenic.

The organisms which will be here considered are: — *Salmonella enteritidis*, the cause of food poisoning, *S. paratyphosa*, the cause of paratyphoid, *S. pullorum*, the cause of bacillary white diarrhea of chicks.

In addition to these, *Salmonella choleræ suis* is frequently associated with hog cholera, and other organisms of the group are known to produce disease in other domestic animals. It will be noted in the following descriptions that these organisms cannot be differentiated from each other on the basis of morphologic or cultural characteristics. The principal means of identification has been the agglutination reaction and differences in pathogenicity. Recent studies have also indicated the possibility of separation on the basis of fermentation of certain carbohydrates.

Salmonella enteritidis

Alternative Name. — *Bacterium enteritidis.*

Synonyms. — It is difficult to list all of the synonyms of this bacillus, as it has been isolated from many sources. Very possibly all of the following are not strictly synonymous. Gaertner's bacillus, *Bacillus of Dunbar*, *Bacillus of Danysz*, Ratin bacillus, Liverpool viruses, etc.

This organism was first isolated by Gaertner from the flesh of a diseased cow, a portion of which had been used for food and had given rise to fifty-seven cases of meat poisoning, with one fatal case. Since that time, this organism and closely related forms have been isolated from a great variety of sources, such as meats which have caused meat poisoning, from the feces, flesh, and blood of animals affected with various diseases, and even from the feces of healthy animals. It is perhaps the commonest cause of food poisoning, frequently mistermed ptomaïne poisoning.

Morphology and Culture. — This organism is an actively motile rod with rounded ends. It is about the size of the colon bacillus. Occasionally long filaments are observed resembling those found in cultures of the typhoid bacillus. The organism does not produce spores or capsules, is gram negative, and stains well with ordinary aniline dyes. It grows best on ordinary alkaline media at blood heat, but it also grows well at room temperature. It is aërobic and facultative anaërobic. It renders broth turbid. Gelatin is not liquefied. Its growth in milk is most characteristic. At the end of a day very little change is to be seen, though the reaction becomes weakly acid. After a longer period, from one to several weeks, the milk becomes yellowish and somewhat transparent, and a strong alkaline reaction is developed. The milk is never coagulated. Indol is sometimes produced in small quantities, but the reaction is usually uncertain. Lactose and saccharose are never fermented, but dextrose is fermented with the development of both acid and gas. This organism is somewhat more resistant to heat than are the other members of the intestinal group. It has been found that 60° C. for an hour is necessary to destroy it, and for shorter periods higher temperatures are necessary. It has also

been found to be more resistant than most forms to the action of the antiseptics used in smoking and pickling foods.

Disease Production. — The smaller laboratory animals all succumb to subcutaneous, intravenous, and intraperitoneal injections and to feeding of this organism. The most susceptible animal is the mouse. Some strains of this organism have also been found capable of producing disease in calves, swine, monkeys, dogs, and cats. However, the virulence of the various strains which have been isolated is exceedingly variable.

The production of disease by this organism when taken into the intestinal tract of man is probably to be ascribed to two causes. The first is the development by the organism, while growing in the food itself of an unusually soluble and powerful endotoxin differentiated from true toxin in that it is exceptionally heat resistant. Even the temperature of boiling water does not greatly reduce its poisonous character. Meat which has been infected and quite thoroughly cooked may give rise to this form of poisoning even though all of the bacteria have been destroyed at this temperature. The quick development of symptoms in persons eating such food is probably due to this endotoxin. Second, this organism may multiply in the intestine or its wall and produce a disease resembling in some respects typhoid fever. This is fatal in a small proportion of cases.

Some of the viruses sold upon the market for the purpose of exterminating rats and mice are probably cultures of this *Salmonella enteritidis* or its varieties. These have been exploited as capable of causing a fatal disease in these rodents, but not pathogenic for man or any of the higher animals. However, in some cases at least, tests have shown that these organisms are capable of infecting higher animals and even man. There does not seem to be any good reason for differentiating this rat virus from *Salmonella enteritidis*.

Very great differences in virulence have been noted in strains isolated from different sources. It is probable that continued residence and growth in a single species of animal may develop an unusually high disease-producing power relative to that particular species, while pathogenicity for some other species may not

be increased. This may account for the very considerable number of distinct strains and varieties of this organism that have been isolated. In many cases these are believed to be distinct species.

Immunity. — No practicable method of immunization of man against this disease has been developed or practiced. Agglutinins specific for the organism causing an infection are developed in the blood and may be used in the diagnosis of the disease. The poisonous product of the growth of this organism is, as has been noted above, an endotoxin and not a true toxin, hence no antitoxins have been developed.

Transmission. — This organism is probably the most important cause of the so-called meat poisoning, also incorrectly known as ptomaïne poisoning. Infection of flesh may be either ante-mortem or post-mortem. Ante-mortem infection may be the result of either primary or secondary infection. The organisms are present in the blood stream and in the tissues in a diseased animal and probably continue to multiply after its death. It can also gain entrance to flesh after the animal has been slaughtered. It has been found in the normal feces. Its presence in such is one of the chief arguments for cleanliness about slaughterhouses. During the slaughtering of animals and preparation of their flesh for the market, every precaution should be used to see not only that the animal is not diseased, but also that fecal material is never allowed to come in contact with the healthy tissues. This means the rigid exclusion of flies and a high degree of cleanliness in all of the operations. Experiments have shown that when *Salmonella enteritidis* is placed upon the surface of fresh meat, it rapidly penetrates to the interior of the tissues even when the meat is stored at a relatively low temperature.

Salmonella paratyphosa

Alternative Name. — *Bacterium paratyphosum.*

Synonyms. — What has been said relative to the synonymy of *Salmonella enteritidis* is even more true of the synonymy of this organism. As here used the name includes *Bacillus paratyphosus*, bacillus of flesh poisoning of the type of Aertryck, *Bacillus*

morbificans bovis, *Bacillus choleræ suis*, bacillus of rat typhoid, of enteritis of cats and organisms isolated from the tissues and feces of diseased animals, and from flesh, sausage, milk, water, ice, etc.

Organisms of this type have been repeatedly isolated from cases of so-called paratyphoid fever in man, a disease resembling clinically the true typhoid fever but differing in that the blood serum does not give the characteristic agglutination reaction with typhoid bacilli, but only with the organism isolated from the feces of the particular individual. This organism has likewise been isolated from many other sources noted above.

Morphology and Culture. — These characters do not differ in any marked degree from those noted for the preceding organism *B. enteritidis*.

Disease Production. — Paratyphoid fever in man is frequently diagnosed as the result of food infection. In some cases it has been definitely shown to be due to the use of contaminated water and milk. In the majority of cases it is probably due to the consumption of infected meat. The disease in man has been attended by a low mortality. It manifests itself as an acute inflammation of the intestines.

The *Bacterium typhi murium*, which, like the bacillus of Danysz, included under the discussion of *Salmonella enteritidis* above, has been exploited as a means of destroying rodents, is to be classified here. While it is not certainly known that this strain is capable of producing disease in man, its use should certainly be attended with much care on account of the possibility of such infection.

Immunity. — It is probable that the disease in this instance is also due to a soluble endotoxin similar to that of *Salmonella enteritidis*. In fact, the principal reason for separating this organism from *S. enteritidis* is the marked difference to be found in the agglutination reaction. Animals immunized by systematic injections of killed or living cultures of *S. paratyphosa* do not develop agglutinins specific for *S. enteritidis;* at least, their blood serum does not agglutinate the former organism in as high dilution as it does *S. paratyphosa*. Practicable methods of immunization in man have not been developed.

Transmission. — Paratyphoid fever is usually transmitted in the same manner as the infection caused by *Salmanella enteritidis*, and the same precautions relative to the use of the flesh of diseased animals and cleanliness about slaughterhouses will apply. In several cases infected water has been definitely shown to be a source of infection.

Salmonella pullorum

Alternative Name. — *Bacterium pullorum.*

Synonym. — *Bacillus pullorum.*

This organism is the cause of bacillary white diarrhea in chicks, one of the most serious diseases of poultry and one which causes great economic losses. Two strains of organisms are known; one, typical, which produces gas, and an anaërogenic strain, which does not. Morphologically and culturally this organism is closely related to the other members of the genus.

The disease occurs in young chicks, causing a white diarrhea, and a "pasting up" which is quite characteristic. The birds become emaciated, and usually succumb, although a few recover. The organisms are excreted in large numbers, and the disease transmitted fom bird to bird by ingestion.

Of special interest is the fact that certain hens are carriers of the infection. The organisms are present in the ovary, and present also in the eggs when laid. Chicks hatched from such eggs already are infected, and soon show symptoms of the disease. From these chicks the disease rapidly spreads to others. It is evident that the elimination of carriers from flocks of hens is most important.

Carriers may usually be recognized by means of the agglutination test. In valuable flocks it is possible to eliminate the carriers and at the same time eliminate the disease by its use.

Subgroup III. Typhoid-Dysentery Group

The organisms belonging to this subgroup include those producing the diseases typhoid fever and bacillary dysentery. The generic name *Eberthella* is sometimes used to designate the motile forms, and Shigella and Flexnerella the non-motile.

Eberthella typhosa

Alternative Name. — *Bacterium typhosum.*

Synonyms. — *Bacillus typhi, Bacillus typhi abdominalis, Bacillus typhosus.*

The organism was first noted by Eberth, who found it in the spleen of persons who died of typhoid fever. It was first cultivated in 1884 by Gaffky. It is difficult, if not impossible, to produce typical typhoid fever in the usual laboratory animals.

This disease is widely spread in temperate and tropical countries, and is constantly present in the United States. It is one of the most important diseases with which the sanitarian has to contend.

Morphology and Culture. — *Eberthella typhosa* is a plump rod 0.5 μ — 0.8 μ × 1.0 μ — 3.0 μ. Usually it occurs singly, occasionally in short chains, sometimes producing long filaments probably to be regarded as involution forms. It is actively motile by means of numerous peritrichous flagella. It does not produce capsules or spores, it stains readily with aniline dyes, and is gram negative.

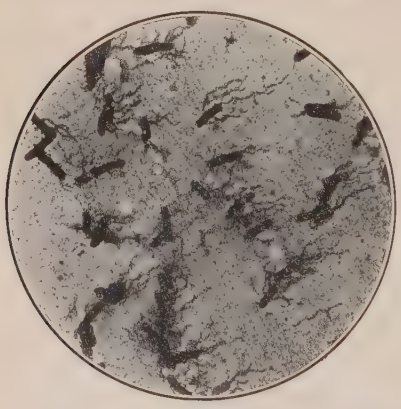


Fig. 162. — *Eberthella typhosa.* (After Günther.)

The organism may be isolated from the blood or spleen of an individual having typhoid fever, and by means of appropriate culture media may be obtained from the feces. It is exceedingly difficult, however, to detect it in water. This has been accomplished only a few times. The colonies upon gelatin are smaller and more delicate than those of *Escherichia coli.* It grows best at a temperature of 37° C., and will grow at room temperature. It is aërobic, but becomes facultative anaërobic in the presence of sugars which it can ferment, such as dextrose. It does not coagulate milk or digest casein.

Disease Production. — The injection or inoculation of *Bact. typhosum* into laboratory animals, such as the guinea pig, produces results differing but little from those obtained by the injection of *Bact. coli*. By means of feeding experiments, it has been found possible to produce the disease in certain monkeys. Accidental infection of man has also occurred in the laboratory from pure cultures. There is no reason for doubt, therefore, as to the causal relationship of this organism to the specific disease. It gains entrance to the body through the alimentary tract, probably multiplies to some extent in the intestinal contents, and invades the lymph system, particularly the clusters of lymph glands called Peyer's patches of the small intestines. These latter become ulcerated and the hemorrhages characteristic of this disease and the occasional intestinal perforation are probably due to these ulcerations. The organism also invades the blood stream so that the disease is to be regarded as a true bacteremia. It is present in very great numbers in the spleen. This latter organ becomes considerably swollen. Occasionally, even after recovery, the infection may persist in some part of the body, as in the gall bladder or in the bones or joints. The organism does not produce a true toxin.

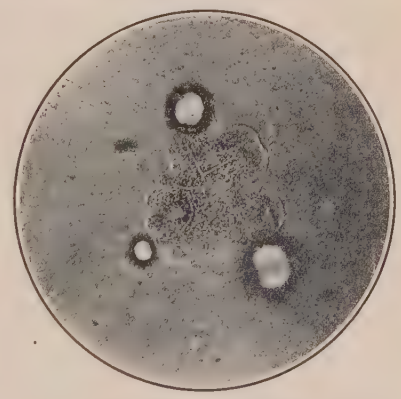


FIG. 163. — Colony of *Eberthella typhosa* on agar. (After Günther.)

Immunity. — An individual convalescing from typhoid shows a considerable degree of immunity, but this disappears gradually, and the disease may again attack the same person. This immunity is believed to be due to the development in the blood of specific opsonins and bacteriolysins. Inasmuch as no toxins are formed by the organism, antitoxins cannot be produced for the treatment of the disease. Antisera have been prepared and tried, but have not proved successful. Within recent years vaccination against ty-

phoid fever has become relatively common. The vaccine is prepared by growing the bacilli on the surface of agar slants, scraping them from the surface of these cultures and suspending them in physiological salt solution, heating to a temperature high enough to destroy the organism. These vaccines have been extensively used for the prevention of typhoid in the armies.

Agglutinins develop in the blood of those who have typhoid fever, and use is made of this fact in carrying out the Widal or agglutination test in the diagnosis of the disease. The method of making this test has already been discussed in the chapter on Immunity. Sometimes the organisms are cultivated directly from the blood of the patient.

Transmission. — Typhoid fever is probably most frequently contracted through drinking water. A city which has a polluted water supply practically always has a high typhoid death rate. The effort made by sanitary engineers and others for the perfection of suitable water supplies has largely been for the purpose of stamping out this disease.

Milk and other foods may also serve as carriers of this organism. Milk is infected only after being drawn from the animal, as the cow itself does not contract typhoid fever. The organism may gain entrance to the milk through the use of polluted water in washing milk vessels or by contact with a person having the disease. It is probable that flies are frequently instrumental in carrying the typhoid bacillus from the dejecta of typhoid patients to food. Within recent years a determined effort has been made to brand the common house fly as the " typhoid fly," inasmuch as it breeds in garbage piles and excreta and undoubtedly is a common carrier of the infection.

Direct infection or contact infection is also relatively common. All of the excretions of the typhoid patient should be carefully disinfected, and care should be used to see that all dishes and utensils used by the patient are boiled.

A very important factor in the spread of typhoid fever is the so-called " bacillus carrier." This term is used to indicate an individual who continues to harbor a pathogenic organism after apparent recovery from the disease. Typhoid bacilli have been

found in the excretions of individuals that have had typhoid fever several years previous to the examination. Such individuals are a real menace to the health of a community, as it is very difficult indeed to guard others from infection. Fortunately only a small number of those who have typhoid fever continue to carry the organisms long after convalescence. Occasionally an individual may have a mild case or a case of walking typhoid. In such cases, evidently the body is sufficiently resistant to prevent the disease from running a typical course, but such individuals are just as dangerous to others as those who have a severe or even fatal case of the disease.

It may be emphasized that typhoid bacilli do not retain their vitality for a long period outside of the body. There is no evidence that they ever multiply in filth or dirt of any kind. They certainly are never carried by air currents. They leave the body only in the excretions, and the only possible way of contracting typhoid is to drink liquids or eat foods which have been infected from such excretions directly or indirectly.

Bacterium dysenteriæ

Alternative Name. — *Shigella dysenteriæ.*

This is the cause of bacillary dysentery in man. The organism was first discovered in 1898 by Shiga in Japan. In 1900 Flexner described a somewhat similar organism in the Philippine Islands. Since that time dysentery has been carefully studied and a considerable number of types of organisms have been isolated. It is entirely possible that several species may be ultimately differentiated, but they may all be grouped together for our present purpose. The bacillus of Shiga is probably the most common and the most important, but the bacillus described by Flexner has also been found in a certain proportion of cases.

Morphology and Culture. — The *Bacterium dysenteriæ* cannot be differentiated from the typhoid bacillus in stained mounts. It stains readily with aniline dyes and is gram negative. Cultural characters are very similar to those of *Eberthella typhosa.* Milk is rendered permanently alkaline. The varieties of *Bacterium dysenteriæ* that have been differentiated have been separated on

the basis of carbohydrate fermentation. As many as fifteen different groups have been created by this method by some authors. The bacillary dysenteries are to be differentiated from the dysentery caused by an amoeba.

Disease Production. — As in typhoid fever, the organism gains entrance to the body by way of the alimentary tract. The intestines, particularly the colon, are inflamed. In some cases the latter may even show necrosis or death of the lining membranes. The organism does not usually gain entrance to the blood and is not common in the internal organs, with the exception of the lymph glands adjacent to the intestines. It is probable that the disease is a toxemia rather than a bacteremia.

Immunity. — It has been found that the Shiga type of dysentery bacillus produces a toxin which is probably responsible for the direct injury to the intestinal walls. The toxin has been prepared in the laboratory by growing the organism in an alkaline broth, and the antitoxin by the systematic injection of such broth into suitable animals. This antitoxin has been found to give favorable results in the treatment and cure of the disease caused by this type of organism. The other types of *Bacterium dysenteriæ* do not seem to produce a true toxin, and no efficient method of treatment by the use of an antiserum has thus far been perfected.

The blood serum of an individual infected with dysentery is found to contain agglutinins specific for the particular type of organism causing the disease. The agglutination reaction has been the principal means of differentiation of the various types of organisms. It enables one by testing the blood serum with each of the types of dysentery bacilli to determine with a fair degree of accuracy the particular type causing the disease in the individual under examination.

Transmission. — Dysentery is transmitted in the same manner as typhoid fever, that is, by water, milk, food, and by direct contact. There is no evidence that the diarrheas and dysenteries of the lower animals are ever produced by these bacteria. Probably carriers are even more important in the transmission of this disease than in typhoid.

Summary

It will be noted that the organisms belonging to the first or colon subgroup show the maximum fermentative powers and the minimum pathogenicity of the organisms of this group. *Escherichia coli* in particular has been found to be most useful in the determination of pollution in water, as its presence is generally held to be indicative of fecal contamination. Care must be used in the differentiation of other members of the colon subgroups which are not equally diagnostic.

The intermediate subgroup is intermediate both in the fermentative and the pathogenic characteristics of its members.

The typhoid-dysentery subgroup shows minimum fermentative power and maximum pathogenicity.

Since these diseases are very commonly transmitted through water, it is appropriate to read the chapter on water and water analysis (Chapter XLVIII) at this point.

CHAPTER XLI

UNDULANT FEVER. ABORTION GROUP

The organisms belonging to this group are sometimes placed in the genus Bacterium or in the genus Alcaligenes. The group is so distinctive in many ways that it seems appropriate to recognize it as a distinct genus Brucella. Two bacterial species are to be here considered; *Brucella melitensis*, the cause of Malta fever, and *Brucella abortus*, the cause of abortion in cattle and other animals.

Brucella melitensis

Synonyms. — *Micrococcus melitensis*, *Alcaligenes melitensis*, *Bacterium melitense*.

This organism is the cause of the disease variously called Malta fever, Mediterranean fever, or undulant fever. It is known from southern Europe and northern Africa, from Mexico, and other countries. In the southwestern United States it is known as goat herders' disease. The organism was first observed in 1887 by Bruce, who regarded the organism as a coccus.

Morphology and Culture. — The *Brucella melitensis* is a short bacillus, often almost spherical. It is about 0.5 μ in diameter, and occurs singly or in short chains. It is gram negative and does not produce spores. Its cultural and physiologic characters are rather negative. It grows on ordinary laboratory temperatures rather slowly. Glucose is fermented little or not at all. Milk becomes somewhat alkaline.

Disease Production. — The disease in man tends to last for a relatively long period. It is characterized by an undulating fever, severe articular rheumatism and by extreme sweating. Although not highly fatal, the disease is a very serious one due to the long course and the extreme debilitation.

The disease may be readily diagnosed by means of the agglutination test.

Transmission. — The disease primarily affects the goat, and is most frequently acquired by drinking the unpasteurized milk from infected goats. The peculiar incidence of the disease may in part be explained by the distribution of goats. It has also been contracted on several occasions by laboratory workers who were handling cultures of the organism. It is of special interest in the United States because of its relationship to the organism next to be considered.

Brucella abortus

Synonyms. — *Bacillus abortus*, *Bacterium abortus*, *Alcaligenes abortus*.

The organism which causes bovine infectious abortion was first described in Denmark by Bang in 1897. The disease is very widespread in Europe and in America and is one of the most important diseases affecting the live stock industry. A similar disease has been reported in swine.

Morphology and Culture. — The organism when first isolated from cattle does not grow well in contact with atmospheric oxygen. When grown in a serum agar " shake " culture the colonies develop in a layer several millimeters from the surface, *i.e.*, the organism is microaërophilic. After cultivation for a time it becomes adapted to aërobic growth.

In general the morphologic and physiologic characters of *Brucella abortus* resemble closely those of *Br. melitensis*. The proof of very close relationship was brought by Alice Evans.

In the cow infection leads to an inflammation of the mucous membrane of the uterus with consequent abortion. A cow which has aborted once or twice may develop an immunity and thereafter carry the calf to full term. The abortion disease has been particularly troublesome in dairy herds, and has led to serious economic losses.

It has been found possible to infect the guinea pig, with the consequent development of arthritis.

Of particular interest is the possible relationship of the abortion

bacillus to undulant fever in man. There have been numerous cases of undulant fever reported in Europe in which there was no probability of infection from the goat, and in which the organism differed in minor respects, particularly immunologically, from the true *Br. melitensis*. To such in some cases the specific name *paramelitensis* was given. Since 1927 it has been increasingly evident that many cases of undulant fever occur in the United States, and that they had been incorrectly diagnosed previously. In the course of two years several hundred such cases were reported from a few states.

These cases of undulant fever have in most cases no history of use of goats' milk. The organisms isolated seem to be related commonly to the strains of abortion bacilli isolated from swine. There have been many cases of infection among employees of packing companies. The evidence is not yet conclusive as to the possible relationship of cows' milk to the disease. The problem is one that is being studied intensively. The evidence points to the advisability of renewed insistence upon adequate pasteurization of milk.

The disease has in the past not infrequently been confused with typhoid or other fevers.

CHAPTER XLII

ACID-FAST OR TUBERCULOSIS GROUP

The principal organisms belonging to the acid-fast group of bacteria are *Mycobacterium tuberculosis*, the cause of tuberculosis in man and animals, *Mycobacterium lepræ*, the specific cause of leprosy, and some non-pathogenic organisms found not uncommonly in soil, feces, butter, milk, etc. The name *acid-fast* (or *acid-proof*) refers to the fact that these organisms are somewhat difficult to stain, but when once stained with the aniline dyes such as fuchsin, they retain the stain with great tenacity, even when treated with relatively strong solution of inorganic acids, such as sulphuric and nitric. These organisms are slender, non-motile rods, never producing capsules or spores, and are gram positive.

Mycobacterium tuberculosis

Synonyms. — *Bacillus tuberculosis*, *Bacterium tuberculosis.*

This is the cause of the disease tuberculosis in man, other animals, birds, and possibly fish and reptiles. The disease in its various forms has been known since ancient times. The different types of tuberculosis, however, differ so much clinically that their relationship was not even suspected until comparatively modern times. The possibility of transferring the disease from one animal to another was demonstrated by Villemin in 1865, but he did not discover the causal organism. The specific cause remained in doubt until the publication of the work of Robert Koch in 1884, in which he described the tubercle bacillus. Koch succeeded not only in demonstrating the presence of this organism in the various lesions of the disease, but also in cultivating it upon artificial culture media. The latter is especially noteworthy, inasmuch

as this organism is not easily cultivated. The presence of acid-fast bacteria in tuberculosis in man and animals, and their close resemblance, led to the assumption that all tuberculosis was caused by the same organism. In 1896 Dr. Theobald Smith noted that it was possible to differentiate the bacteria causing tuberculosis in cattle from those characteristic of the disease in man. Even earlier it was shown that the bacteria producing avian tuberculosis are distinct. Dr. Koch directed his attention to investigations along this line, and in 1901 read a paper before the International Tuberculosis Congress which met in London, asserting that the bovine and human tubercle bacilli are distinct, and that the probability of transmission from the bovine to the human is extremely remote. This ran counter to the general opinion among bacteriologists and sanitarians, and led to a vast amount of work on this subject. At the present time, the existence of three varieties of the tubercle bacillus is generally admitted, namely the avian, the human, and the bovine. These have many characteristics in common, hence they may be discussed together. Whether or not the one type can be transformed into another is a disputed question. There is no conclusive evidence that such is possible.

Tuberculosis is one of the most important of the bacterial diseases affecting men; formerly it caused more deaths than any other single disease. In the United States alone, over 110,000 deaths occur annually from this disease. It is also widely distributed in cattle. In the United States it is quite common in both dairy and beef herds. In some localities from ten to twenty-five per cent or even more of the milk cows have been found to be affected with the disease. Swine also contract tuberculosis when fed milk from tuberculous animals or when allowed to run in the feed lot together with tuberculous cattle. Tuberculosis in chickens has been reported from a considerable number of localities in North America.

Morphology and Culture. — *Mycobacterium tuberculosis* is a slender rod with rounded ends. It may be straight or somewhat bent. It measures 0.2–0.5 μ × 1.5–3.5 μ. Occasionally much longer filaments are found. It is usually single, almost never occurring in chains. The protoplasm often takes the stain irreg-

ularly, giving the cell a beaded appearance. It does not produce spores or capsules, and is non-motile. Involution forms, such as branched and club-shaped rods, are sometimes observed. The organism stains with difficulty with the ordinary aniline dyes. It is generally necessary to heat the dye or to allow it to remain for a long time in contact with the organism before it stains sufficiently for observation. When once stained, however, the application of acids will not decolorize the cells. This character is of value in the diagnosis of the disease, as it renders recognition of the organism in sputum or tissues possible. Some authors believe differentiation of bovine and human tubercle bacilli is possible in stained mounts; that the bovine are shorter, straighter, and thicker than the human, and that the latter more frequently show beading of the cell. This is, however, denied by many present investigators, as the morphologic characters seem to depend quite as much upon the conditions under which the organism is grown as upon the animal from which it is obtained.

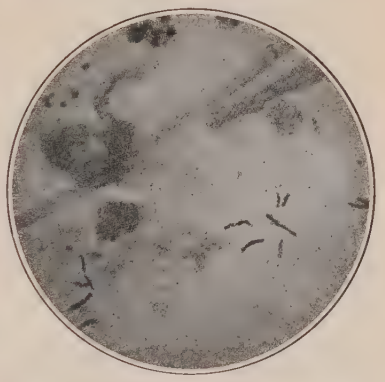


FIG. 164. — *Mycobacterium tuberculosis* in sputum. (× 1000.)

The *Mycobacterium tuberculosis* is isolated from the lesions of the disease with a considerable degree of difficulty, inasmuch as it does not grow readily upon most of the culture media, at least at first. Whenever it occurs mixed with another organism, animal inoculation must generally be resorted to in order to secure a pure culture, the organism then being isolated from the characteristic lesions. Pure cultures are secured by rubbing bits of infected tissue containing the nodules or tubercles over the surface of coagulated blood serum and incubating at blood heat. The organism does not usually show any growth for a period of a week or more. The colonies appear at first as tiny grains, barely visible to the naked eye. These gradually enlarge and may become confluent.

It also has been found possible to treat mixtures of tubercle bacilli and other organisms, as in sputum, with strong alkalies which will destroy the other bacteria, but leave the tubercle bacillus alive. After cultivation for a time upon protein medium, transfers may be made to glycerol agar. Upon this medium it is usually possible to differentiate the bovine and human tubercle bacilli from each other. The bovine bacillus develops characteristically as discrete colonies which rarely fuse to form a continuous layer over the surface. The human bacillus, on the contrary, grows much more luxuriantly; the colonies fuse and the growth becomes considerably wrinkled. On glycerol broth, the growth appears as a more or less heavy pellicle upon the surface of the medium. It is easily broken to pieces and settles to the bottom when shaken.

FIG. 165. — *Mycobacterium tuberculosis*, cultures of human and bovine types on glycerol agar. 1, bovine type; 2, human type. (After Griffith in report of Royal Commission.)

The tubercle bacillus is aërobic. It has an optimum temperature of about 37° C. and a limited growth temperature range. After long cultivation upon artificial media, growth may occur at somewhat lower temperatures. The thermal death point for the organism is 60° C. for 20 minutes. Considerable confusion exists in the literature relative to this thermal death point because of difficulties in the method of determination. Its accurate determination is of particular interest in the pasteurization of milk. The work of members of the Hygienic Laboratory of the United

States Public Health Service seems to indicate that pasteurization of milk at the temperature noted above will certainly destroy the tubercle bacilli present, provided the vessel in which pasteurization is carried out is closed so that no scum can form on the surface of the milk. Sunlight is an active disinfectant. When the tubercle bacillus is dried in sputum, it is quite resistant to desiccation and to the sun's rays.

The principal physiologic character which has been suggested as useful in the differentiation of human and bovine tubercle bacilli is that of the reaction produced in glycerol broth. This has been carefully worked out by Theobald Smith, who has come to the conclusion that in glycerol bouillon, the human bacillus brings about a permanent acid reaction, while the bovine bacillus causes the reaction to become alkaline in the course of time.

Disease Production. — Tuberculosis is a disease to be classed among chronic infections. V. A. Moore states, "It does not destroy life by acute toxemia, but by a chronic and long-continued systemic poisoning and by the morbid changes brought about through the localization of these lesions in the organs necessary to life."

The disease gets its name from the production of the characteristic nodules or tubercles in the tissues affected. The organism sets up a local inflammation in any tissue to which it gains entrance and brings about at that point a gradual formation of several more or less concentric layers of cells. The structure of the nodule is practically the same no matter what the tissue affected.

Tuberculosis is easily transmitted to laboratory animals. In general, the bacillus of bovine origin is more virulent than that from the human. For example, a subcutaneous injection of guinea pigs with bovine bacilli generally causes death in less than five weeks, while those inoculated with the human bacillus usually live a longer period. Intraperitoneal injections of bovine bacilli are commonly fatal in from one to three weeks, while the human bacillus generally produces death in from ten days to five or six weeks. Even more marked differences are to be noted in the rabbit. Bovine bacilli injected intravenously into this animal generally produce death within three weeks, while with human

bacilli, rabbits commonly live for several weeks and frequently recover completely. It is also found that human bacilli rarely or never produce tuberculosis when injected into cattle, even into calves. This character is the most important and probably the most reliable of the methods used in differentiating the two organisms. It has been found, however, that both human and the bovine tubercle bacilli can produce tuberculosis when fed to the monkey or injected into its body.

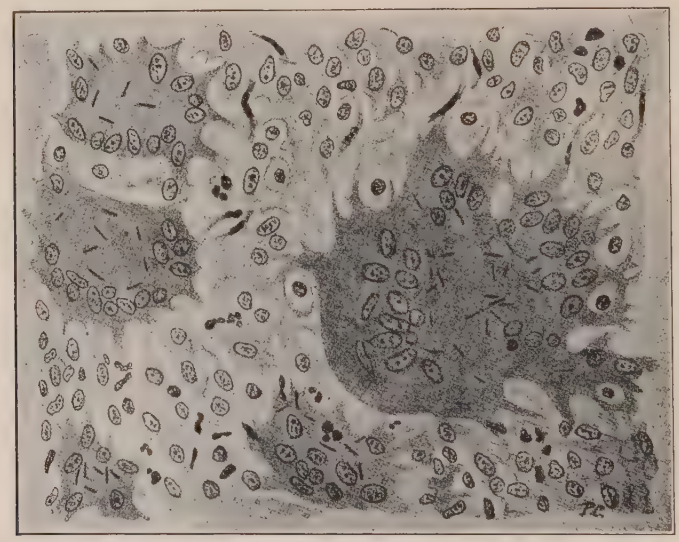


FIG. 166. — Section of a tubercle of the intestinal wall, showing the bacilli and the giant cells. (After Chausse.)

Practically any part of the body may be affected in tuberculosis. The lymphatic system is usually involved. As the nodules enlarge they often coalesce and the interior becomes cheeselike in consistency (undergoes caseation). In some cases the nodules may become surrounded by a capsule of fibrous tissue, quite efficiently preventing the escape and spread of the organism. In other cases the nodules may ultimately become calcified. These calcareous nodules are not infrequently found in healed tuberculous areas. Within the body the bacteria are probably most

frequently spread by the lymphatics, sometimes also by the blood stream.

In the human the tubercle bacillus most frequently attacks the lungs (consumption). Tuberculous infection of the glands of the neck (scrofula), of the bones and joints, of the alimentary tract and abdominal organs, such as the liver, spleen, and kidneys, and even of the brain covering (tubercular meningitis) or of the skin (lupus) is also caused. In cattle the nodules appear most frequently upon the membranes lining the peritoneal cavity and covering the intestines. They are not infrequent in the lungs and accompanying lymph glands. Of particular importance is the occasional development of tuberculosis in the udder of the cow. The percentage of tuberculous udders among tuberculous cows is not certainly known. Some authors believe it to be less than 1 per cent, others estimate it as high as 5 per cent. Swine are most commonly infected in the lymph glands of the neck (scrofula) and in the abdominal organs.

Immunity. — The tubercle bacillus does not form true toxins, hence antitoxins cannot be produced. Poisonous substances, possibly of the nature of endotoxins, are formed. They are probably released in large part only upon the dissolution of the bacterial cell. Satisfactory methods of immunization by the use of vaccines, antisera, etc., have not been developed, although a vast amount of work has been done in an effort to develop such products. There is probably a considerable degree of natural immunity to tuberculosis, for a large percentage of individuals infected with this organism recover from the disease. This is true both in man and animals. It has not been shown, however, that those who have recovered in this manner are resistant to new infection. To what immunity in this disease is due is not certainly known. It is probably in part opsonic. Some use has been made within recent years of tuberculin injected subcutaneously in minute quantities into those affected with tuberculosis in an effort to stimulate the production of antibodies and the development of immunity.

Diagnosis. — Tuberculosis is diagnosed usually by one of three methods, which may be designated as the staining method, animal inoculation, and tuberculin test.

Diagnosis by Staining Method. — Use may be made of the acid-fast characters of the tubercle bacillus in recognizing it in stained mounts. Sputum, for example, from an individual suspected of having tuberculosis may be spread upon a glass slide, dried, and fixed, then stained with hot carbol fuchsin for several minutes. This stains both the tubercle bacilli and all other organisms and cells present in the sputum. The mount is treated with acid alcohol or solutions of sulphuric or nitric acids. This removes the color from all organisms not acid-fast. After washing in water, the counter stain may be made by means of methylene blue. Upon examination under the microscope, the tubercle bacilli are found to be bright red in color, all other bacteria and cells being stained blue. This staining method cannot be used in the recognition of tubercle bacilli under all conditions, inasmuch as there are many other acid-fast bacteria that are not uncommonly found in milk, feces, and soil, etc. These rarely if ever are present in the sputum. The recognition of acid-fast bacteria in this material is almost certainly diagnostic of tuberculosis.

Diagnosis by Animal Inoculation. — The injection of material containing tubercle bacilli into the guinea pig will result in the development of tuberculosis in this animal within a few weeks. The organism may be isolated from the characteristic nodules of the diseased animal. The most certain method of detecting the presence of tubercle bacilli in milk is by means of animal inoculation.

Diagnosis by Tuberculin. — Tuberculin is the name applied to a suspension of dead tubercle bacilli or a solution of their products. It has been prepared in many different ways, some types of tuberculin being used only for diagnostic purposes, others, as has been noted above, in the treatment of the disease. That most commonly used is Koch's Old Tuberculin. This is prepared by inoculating flat-bottomed flasks containing 4 per cent glycerol broth with a culture of *Mycobact. tuberculosis*. Care is used in making the transfer to make sure that the organism floats on the surface of the medium. It spreads out in growth until a heavy film is developed, which finally becomes much wrinkled. The flask is incubated until the maximum amount of growth has developed,

usually for about eight weeks. The contents of the flask are then sterilized by heat and evaporated down to about one tenth of the original bulk. This kills the bacteria. The mixture constitutes tuberculin. In some cases this is passed through a porcelain filter to remove the bacterial cells. The tuberculin then contains only the soluble products of the bacterial growth. Other types of tuberculin are prepared by drying, grinding, and extracting the tubercle bacilli with water, and still others by precipitation with alcohol and re-solution of the precipitate in water.

Tuberculin is used in several ways in the diagnosis of the disease. The test as commonly applied to cattle is to inject the equivalent of 0.25 cc. of Koch's Old Tuberculin subcutaneously. The temperature of the animal is taken at a sufficient number of intervals before the injection, to ascertain the normal for the individual. Beginning six or eight hours after the injection, the temperature is again taken every two hours during the remainder of twenty-four hours. An animal having tuberculosis will show a fever reaction which reaches its maximum intensity usually in ten to eighteen hours after injection and should show at least 1° F. rise in temperature. Just why the injection of tuberculin into tuberculous animals should give rise to a fever reaction but not do so in the normal individual has not been explained satisfactorily. The best solution that has thus far been offered seems to be to regard it as one of the manifestations of the phenomenon of anaphylaxis. It is easily proved that the presence of the tubercle bacilli in the body renders the tissues very sensitive to the injected tuberculin, and the fever reaction is one of the manifestations of this sensitiveness. When properly used, the method is very reliable. By its means tuberculosis in cattle may be readily detected. A similar method may be used for the diagnosis of tuberculosis in the human, but this is not usually carried out on account of the severity of the reaction.

It was noted above that all of the tissues of the body of an individual having tuberculosis are sensitive to the tuberculin. This fact has led to the development of several tuberculin tests for use in man. The cutaneous tuberculin test of Von Pirquet has been much used with young children. The skin on the under

side of the forearm is washed with ether and a drop of tuberculin is applied. The skin is slightly scarified and the tuberculin rubbed in by means of a bit of cotton. In an individual having tuberculosis a papule somewhat resembling that produced in smallpox vaccination will appear. It has also been noted that when a drop of purified tuberculin is carefully placed upon the surface of the eye of a person having tuberculosis, the eye becomes inflamed within five or six hours, the inflammation disappearing within two or three days. The eye of a normal individual shows little or no reaction.

Tuberculosis in cattle and in fowls may be diagnosed by means of the intracutaneous or intradermal tuberculin test. In this method the tuberculin is injected into the skin (not under). Its resorption is slower, and if the animal or bird is infected a marked swelling develops. The skin at one side of the tail is usually chosen for the test in cattle, and the wattles in the fowl.

Tuberculosis is generally classified into two types, open and closed. In open tuberculosis the bacteria are constantly leaving the body, because the lesions lie near some of the channels of exit. In closed tuberculosis the bacteria do not leave the body, because the lesions do not open into any channel communicating with the surface.

Paths by which Tubercle Bacilli leave the Body. — Tubercle bacilli are commonly present in the mouth and sputum of the human affected with pulmonary tuberculosis. This is probably the commonest channel by which the organism leaves the body in this disease. Coughing has been shown to throw off so-called infectious droplets, that is, particles of saliva or sputum containing tubercle bacilli. These usually settle quickly, but may remain in suspension for a sufficient period to allow of their inhalation by others in the immediate vicinity of the patient. The organism is thrown off in great numbers in the sputum, and very great care should be used with a tuberculous patient to see that the sputum is disinfected or burned. The tubercle bacilli within the sputum can retain their vitality for a considerable length of time even when the sputum is dried, ground, and blown about as dust. More important than either infectious droplets or dried sputum in the spread of the disease is the use of infective drinking

vessels or utensils for human food. The use of the public drinking cup has been prohibited in several of the states, due to the danger of the transmission of the disease in this manner. Tubercle bacilli are also swallowed and appear in the feces. Probably this is not a very common means of spreading the disease in man, however. In cattle tubercle bacilli coughed from the lungs are usually swallowed, therefore both pulmonary and intestinal tuberculosis in these animals causes the appearance in the feces of a considerable number of the organisms. Probably most of the organisms leave the body of the cow in this manner. This fact is responsible in large measure for tuberculosis in swine allowed to run with cattle. Tubercle bacilli are frequently ingested under these conditions. The presence of tubercle bacilli in milk of tuberculous animals is an exceedingly important problem, as this would appear to be the commonest method of transmitting the disease from cattle to man. It has already been noted that tuberculosis of the udder occurs in a small percentage of the cows having tuberculosis. Some authorities believe that the tubercle bacilli may gain entrance to the milk even though the udder is not tuberculous. This must be regarded at the present time as not proved. Probably the commonest source of tubercle bacilli in milk is not from a tuberculous udder, but from bacilli excreted in the feces. When the bacteria are leaving the body in this manner, they must occur commonly upon the surface of the body and upon the udder so that they easily gain admittance to the milk during the process of milking.

There is no evidence that tuberculosis is inherited either in man or in animals. Occasionally an individual may be born with tuberculosis, but an examination will show the uterus of the mother or the placenta to be affected as well. It is true, however, that different families show variations in resistance to the disease. This lack of resistance, taken together with the abundant opportunity for infection in such cases, explains the fact that tuberculosis is generally regarded as "running in families." It has been found possible to build up herds of cattle free from tuberculosis by removing the newborn calves from their tuberculous mothers and feeding them only upon milk known to come from animals not infected with the disease or that has been pasteurized.

Portals of Entry in Tuberculosis. — There seems to be little question but that tuberculosis in man is frequently acquired by inhalation of the tubercle bacilli. The second most common method of infection probably is by ingestion. Until recent years it has been generally conceded that tuberculosis of the lungs must be due to inhalation of the tubercle bacilli, and tuberculosis of the abdominal organs, particularly the intestines, due to ingestion. It has been shown, however, that when tubercle bacilli are fed in considerable numbers to a young animal, they may frequently be demonstrated within a few hours in the thoracic duct of such an animal. In other words, the organism seems to be able to penetrate the intestinal wall of a young animal under favorable conditions, first gaining entrance to the lymph channels and then to the blood stream, and so infecting the lungs. At present there is marked disagreement among pathologists as to the relative importance of inhalation and ingestion. Some go so far as to say that infection by inhalation rarely if ever occurs, while others believe that ingestion is relatively unimportant. Diseased tonsils also offer opportunity for infection of the neighboring lymph glands and the consequent production of scrofula. Occasionally tuberculosis may follow direct inoculation of the skin. This has been reported in a few instances in butchers and veterinarians.

Intertransmissibility of Bovine and Human Tuberculosis. — One of the most important problems which the bacteriologist has been called upon to determine is whether or not bovine tuberculosis is transmissible to man. It has been shown above that the human type of bacillus rarely causes disease when injected into cattle. If it is also true that bovine tubercle bacilli cannot be transmitted to man, many of our sanitary regulations concerning the use of milk and meat containing bovine tubercle bacilli might be relaxed. Until recently we have had but little direct evidence upon this problem. Within recent years, however, a very considerable number of cases of tuberculosis in man, particularly in children, have been found to be caused by bovine bacilli. The following table, adapted from a paper by Parke and Krumwiede, summarizes the results from cases that have been carefully investigated.

Diagnosis	Adults 16 Years and over		Children 5 to 16 Years		Children under 5 Years	
	Human	Bovine	Human	Bovine	Human	Bovine
Tuberculosis of lungs	644	1(?)	11		23	1
Lymph glands and neck scrofula	27	1	36	21	15	21
Abdominal tuberculosis . . .	14	4	8	7	9	13
Generalized tuberculosis of alimentary tract	6	1	2	3	13	12
Generalized tuberculosis . . .	29		4	1	43	5
Tuberculosis of bones and joints	27	1	38	3	26	
Other types	30	2	18	1	86	13
Total	777	10	117	36	215	65

A study of this table seems to leave no reasonable doubt but that a certain percentage of cases of tuberculosis in the human are caused by the bovine bacillus. It is evident also that the disease is common enough in children under sixteen years of age to justify all reasonable precautions against the use of tuberculous meat and milk. The probability of adults contracting bovine tuberculosis seems to be rather remote.

Mycobacterium lepræ

Synonyms. — *Bacillus lepræ, Bacterium lepræ.*

Mycobacterium lepræ is the cause of leprosy in man. The organism was discovered by Hanson in 1874, and described at greater length by Neisser and Hanson in 1880. The disease is a common one over a large portion of Asia and nothern Europe (particularly the Scandinavian countries), in certain of the Pacific islands, and is occasional in the United States. Most of the cases in the latter country are imported from Europe.

Morphology and Culture. — *Mycobacterium lepræ* closely resembles *Myc. tuberculosis* in its morphology. It is a slender rod some-

times as much as 6 μ in length. It is easily stained, non-motile, and does not produce spores or capsules. The organism is distinctly acid-fast, but stains somewhat more readily than *Mycobacterium tuberculosis*. It has not been satisfactorily cultivated upon artificial media until recently. Duval and Cornell have succeeded in cultivating the organism and producing experimental lesions and even death in monkeys inoculated.

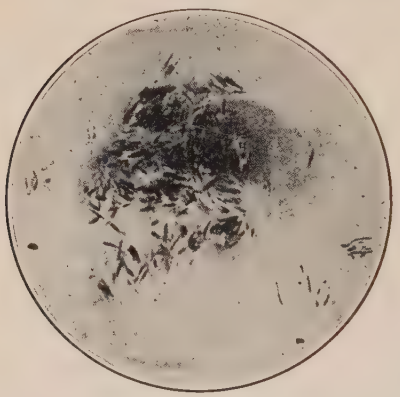


Fig. 167. — *Mycobacterium leprœ*. (After Günther.)

Disease Production. — The disease resembles tuberculosis in some respects. It manifests itself in several forms. In some cases the principal symptoms are nervous. Certain portions of the body lose the power of feeling; they are said to become anæsthetized. In other cases nodules resembling those of tuberculosis develop in the subcutaneous tissues. The disease frequently progresses slowly. Numerous cases have been observed of many years' standing. Recovery is exceptional, but is known to occur.

Immunity. — Practicable methods of immunization and treatment by bacteria or their products have not been developed.

Transmission. — The means by which leprosy spreads is not certainly known. The organism is generally present in considerable quantities in the nasal mucosa of those who have the disease. It is not impossible that the nose is the infection atrium. Some investigators have believed the disease to be spread by mosquitoes, fleas, bedbugs, or other insects. The evidence is very inconclusive.

Other Acid-fast Organisms

One additional acid-fast organism is known, the *Mycobacterium paratuberculosis*, which is the cause of Johne's disease in cattle. This disease is a chronic dysentery.

It has already been noted that acid-fast bacteria are not uncommon in the intestines, soil, milk, etc. These resemble *Mycobacterium tuberculosis* rather closely in their morphology, but develop much more luxuriantly upon the culture media, particularly when the culture is kept at room temperature. It is sometimes impossible to differentiate between these organisms and *Mycobacterium tuberculosis* except by animal inoculation, and even this method must be used carefully inasmuch as the injection of considerable quantities of normally non-pathogenic acid-fast organisms may cause in the tissues of susceptible animals a development of nodules resembling those of tuberculosis. Isolation of the organisms, however, from these nodules reveals distinct differences, so that in practice little difficulty is actually encountered. Acid-fast bacteria have been isolated in a considerable number of cases from butter. Except for the danger of confusion with the true tubercle bacillus, these organisms do not have any economic importance.

CHAPTER XLIII

PATHOGENIC SPORE-PRODUCING ORGANISMS

The pathogenic bacteria which produce spores are relatively few in number. They may be divided for convenience into those which are aërobic and those which are anaërobic.

The *aërobic spore-producers* constitute what may be termed the *anthrax group* of bacteria. The organisms of this group are gram-positive bacilli, capable of liquefying gelatin. The group is very

Fig. 168. — *Bacillus anthracis*. Bacilli in the blood, showing capsules.

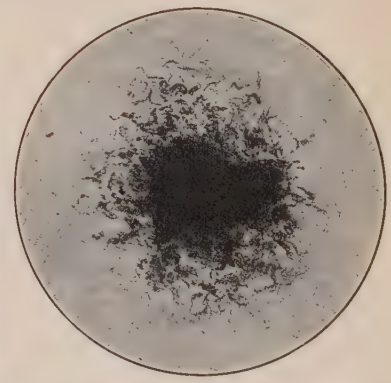


Fig. 169. — Agar colony of *Bacillus anthracis*. (After Günther.)

closely related to the *Bacillus subtilis* or hay bacillus group of organisms responsible for the decomposition of proteins. *Bacillus anthracis*, the cause of anthrax in certain domestic animals, occasionally in man, is the only pathogenic form. The disease is a bacteremia causing high fever and usually death within a few days in the animals infected, principally cattle and sheep. The disease may be transmitted to man from handling hides from cattle

that have died of the disease, or in sorting wool in which the spores of the organism may be present. When inoculated under the skin, as through a wound, it produces what is known as a malignant carbuncle which may heal spontaneously or may result in the general invasion of the blood stream and cause death from bacteremia. The inhalation of the organism may produce the so-called woolsorter's disease, a very acute and malignant type of pneumonia. During the Great War there were some cases of anthrax in soldiers due to the use of shaving brushes made from hair that had not been properly sterilized. Inasmuch as this is a disease primarily of animals, one which is not very common and not often transmitted to man, it will not be discussed further.

Group of Anaërobic Spore-producing Bacteria

This group includes a considerable number of forms. Many of them are not uncommon in the soil, most of them are non-pathogenic. A few, however, are capable of producing diseases. Among these are *Clostridium tetani*, producing tetanus or lockjaw; *Cl. botulinum*, causing a type of meat poisoning; *Cl. œdematis*, the cause of malignant œdema; *Cl. chauvœi*, the cause of black leg in cattle; and *Cl. welchii*, which has been found in connection with a few cases of disease in man as a probable cause of gaseous œdema. *Cl. tetani* and *Cl. botulinum* are of importance in the causation of disease in man. The discussion will be limited to these forms.

The organisms of this group are united because of their failure to develop in the presence of an abundant supply of oxygen. All produce spores, and most of them develop gas from carbohydrates and sometimes from proteins.

Clostridium tetani

Clostridium tetani is the cause of tetanus or lockjaw in man and certain of the domestic animals, particularly the horse. It was discovered in 1889 by Nicolaier, who found it in pus from animals that had died as a result of the subcutaneous inoculation of a suspension of rich garden soil. He succeeded in growing the organism in artificial media, but could not get it in pure culture.

Kitasato in 1889 secured pure cultures under anaërobic conditions and proved experimentally that it was the specific cause of the disease tetanus. Together with Veyl in 1890 he demonstrated the production of a specific toxin in culture media. This organism is not uncommon in the alimentary tract of herbivorous animals, particularly the horse, is frequent in rich garden soil, and is practically always present in street dust. It is not certainly known whether it can multiply in nature outside of the body, but this seems to be probable. It is perhaps to be regarded as a normal saprophyte that is only capable of producing disease under exceptional conditions.

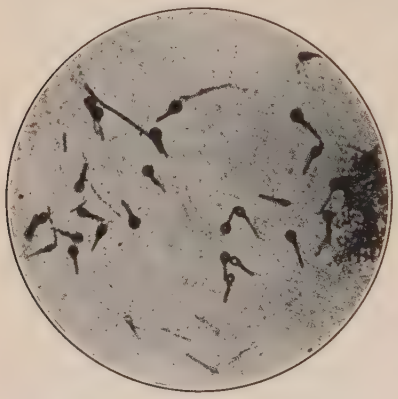


FIG. 170. — *Clostridium tetani.* (After Günther.)

Morphology and Culture. — *Clostridium tetani* is a long, slender motile rod 0.5 × 2.0–5.0 μ. It usually occurs singly, occasionally it appears in short chains. Capsules are not formed. Spores are produced abundantly under almost all conditions. These spores are several times the diameter of the rods upon which they are borne. They resemble in some degree those of *Cl. putrificum*, already described. They occur at the ends of the rods, giving a characteristic drumstick appearance. The organism stains readily and is gram positive. The fact that this organism is an obligate anaërobe renders it somewhat difficult to cultivate. It was first isolated by producing tetanus in experimental animals, inoculating a broth culture from the pus of the wound and heating to 80° C. for one half hour. The spores of the organisms will resist this temperature, while other bacteria present will be destroyed. An inoculation from this broth into agar or gelatin kept under anaërobic conditions will usually show development of the tetanus bacillus. The organism grows readily upon most of the culture media. A stab culture in gelatin shows an arborescent, radiating growth

along the line of stab. Gelatin and blood serum are liquefied and milk is coagulated by the production of acid.

The optimum growth temperature is blood heat, although the organism grows well at room temperature. It develops only when oxygen is almost wholly excluded from the medium. It will also grow under aërobic conditions when mixed with aërobic bacteria. The spores are quite resistant to drying and to other unfavorable conditions. In some cases the resistance to heat is so great that exposure to live steam for over an hour is not sufficient to destroy them certainly.

Disease Production. — The disease is most common in man and the horse. It is ordinarily the result of wound infection. The organism gains entrance to a wound, particularly one which is relatively deep and heals or closes superficially, and there produces its characteristic toxin. This is taken up by the motor nerve endings, passes along the nerve trunk to the central nervous system; probably in part also passes to the blood stream and is carried by this means to certain portions of the central nervous system. The nerve cells are injured, and the muscles of the body, particularly those of the part affected, become rigid. They are said to go into a state of *tetanus*. The bacillus rarely gains entrance to the circulating blood and does not develop in the tissues to any great distance from the point of inoculation. The growth of the organism, the development of the toxin, and the absorption of this toxin by the nerve cells is so slow that the period of incubation in man averages about nine or ten days. In the horse it may be somewhat longer. The disease is not contagious. The only common means of infection, as has been stated, is through a dirty wound. The puncture from a rusty nail, for example, is infective not because of the rust, but because of the dirt containing spores of the tetanus bacillus which is apt to be present upon its surface. These spores are carried deep into the wound by the rough surface of the nail, and are left there upon its withdrawal. Bright or clean instruments are not as apt to transmit the disease because there is greater opportunity for the organism to be brushed aside by the skin and because they do not ordinarily have the specific organism upon their surfaces. An important point to be noted is the fact

that when the spores of the tetanus bacillus are obtained entirely free from their characteristic toxin by repeated washing, pure cultures may be injected subcutaneously into animals without producing the disease. The organism finds its most favorable conditions for development when associated with dirt in wounds, and particularly with some of the pus-producing bacteria.

Immunity. — Tetanus is a toxemia. It is possible, therefore, to produce antitoxins for the disease. The toxins are developed readily in culture flasks of broth kept under anaërobic conditions. After incubating for a week or more, the broth is filtered and the bacteria removed. The toxin may be concentrated from the broth by means of precipitation by ammonium sulphate. By such methods toxins have been secured so potent that 0.00000025 gm. is sufficient to kill a white mouse. It has been shown that the toxin has two poisonous constituents, one termed *tetanolysin* that is capable of destroying red blood corpuscles, the other *tetanospasmin*, which has a particular affinity for the nerve cells and which is the immediate cause of the characteristic symptoms of tetanus. The antitoxin is prepared by the injection of increasing doses of the toxin into a healthy horse. After a time the animal may develop a high grade of immunity. Blood may then be drawn, the blood serum removed and utilized as a means of preventing the disease in man. It is now customary for physicians to inject antitoxin as a preventive whenever there is evidence that the tetanus bacillus may have gained entrance into a wound. Inasmuch as the symptoms of tetanus only become apparent after a considerable degree of the damage has been done, it is evident that the injection of antitoxin into an individual already showing these symptoms cannot undo the damage already done. In other words, while tetanus antitoxin is an excellent prophylactic, and its injection will practically insure the prevention of tetanus, its usefulness as a curative agent is not so evident.

Transmission. — It has already been noted that tetanus is an example of a non-contagious infectious disease. The infection occurs almost invariably directly through the skin, as the result of a relatively deep or dirty wound. Sometimes the infection atrium is difficult to determine.

Clostridum botulinum

Synonym. — *Bacillus botulinus.*

This organism is one of the frequent causes of meat and food poisoning in man. The disease is generally termed botulism from the Latin *botulus* (sausage). The organism was first isolated by Van Ermengen in 1896 from a sausage which he believed to be the cause of an outbreak of food poisoning. Poisoning caused by this organism should not be confused with that caused by *Bacterium enteritidis* or with the so-called ptomaïne poisoning.

Distribution. — Outbreaks of poisoning of the type of botulism have been repeatedly reported from Europe, particularly as the result of eating meat foods and sausages. In the United States practically all of the cases reported have been from the eating of imperfectly preserved canned foods. A considerable variety of these have been found to be at fault. Cases of poisoning from canned asparagus, canned beans, and ripe olives have been reported. Most of the cases have been from the Pacific coast.

Morphology. — The *Clostridium botulinum* is a relatively large bacillus, usually single, occasionally in pairs or chains. It is .9–1.2 $\mu \times 4$–6 μ. It is actively motile by means of from 4 to 8 peritrichous flagella. Oval or elliptical spores are produced, one near the end of each cell. The organism stains readily and is gram-positive.

Culture. — Care must be taken to exclude oxygen in the growth of *Clostridium botulinum.* It grows fairly readily in ordinary cultural media.

Physiology. — The organism is an obligate anaërobe. Some strains grow best at room temperatures, others at body temperatures. Apparently there are several distinct varieties of this organism which show minor differences in physiological reactions. Gas is generally produced from dextrose but not from sucrose or lactose.

Pathogenesis. — When growing under favorable conditions, the *Clostridium botulinum* produces a highly potent toxin. Usually this is accompanied by the formation of malodorous substances, particularly butyric acid. Usually food in which this organism

has been growing can be detected by having an "off" odor. Such foods must be carefully avoided, as even a small portion of such food, sometimes not more than a mere taste has proved sufficient to be fatal.

The toxin produced by growing the organism in culture media is quickly fatal to laboratory animals when fed or injected. It is also fatal frequently to man. In man the symptoms include lack of coördination of the eyes (that is, double vision) and difficulty in control of the muscles governing swallowing.

Until recently it has been believed that the organism itself (free from toxin) when it has been introduced into the animal body is non-pathogenic, but more recent work seems to show that the organism when injected or fed to certain animals is capable of producing serious or even fatal results.

Limber-neck of chickens may be caused by feeding food containing the organism or its toxin. The birds are partially paralyzed, resting the tip of the bill upon the ground, and showing lack of ability to hold the head erect.

Immunity. — *Clostridium botulinum* produces one of the most powerful toxins that has been described; 0.00005 gm. has been found to produce death in three to four days when injected subcutaneously into a guinea pig, and ten times this amount will destroy a rabbit. It is to be sharply differentiated from the toxins of the tetanus and diphtheria bacilli in that it can produce characteristic symptoms when introduced by way of the alimentary tract, whereas the toxins of both the tetanus and diphtheria bacilli are rendered innocuous by the digestive juices. This toxin has the various characteristics which have been listed by Ehrlich as determining a true toxin. It is easily destroyed by exposure to light and air, and heating to a temperature of 80° C. renders it inactive. It is evident therefore that the heat of cooking is sufficient to destroy the toxin.

Inasmuch as this organism produces a toxin, it is possible that the corresponding antitoxin could be used in treating the disease. In animals it has been used effectively in curing the disease; it may possibly be of value in man. There appear to be at least two distinct strains of *Clostridium botulinum* whose toxins are specific; it is

necessary therefore to utilize the antitoxin homologous to the organism causing the disease.

Since this organism has been found to infect man most frequently from canned foods, it is of interest to note that the disease has resulted both from commercially canned and home-canned foods. It is highly important therefore that canned foods be adequately sterilized, and care should be used not to consume foods which are "off" in odor.

CHAPTER XLIV

VIBRIO AND SPIROCHETE GROUPS

One organism only, the *Vibrio choleræ*, or spirillum of Asiatic cholera, will be discussed in this group. Related organisms are known to produce disease in animals, and certain related non-pathogenic forms have been isolated from water.

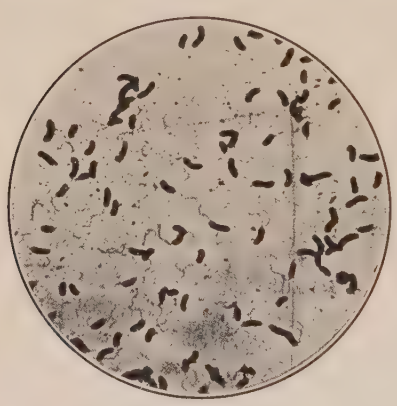


Fig. 171. — *Vibrio choleræ*. (After Günther.)

Vibrio choleræ

Synonyms. — *Spirillum choleræ*, *Spirillum choleræ asiaticæ*, *Microspira comma*.

This organism is the specific cause of Asiatic cholera of man. It was first described in 1883 by Koch, who found it in great numbers in the so-called rice water stools of cholera patients. The disease is constantly present in certain parts of India and in China, and on several occasions has spread in epidemic form to other parts of the civilized world, particularly to Europe.

Morphology and Culture. — The Asiatic cholera spirillum is a slightly curved rod. Filamentous and involution forms are not infrequently observed in culture media. A single polar flagellum is produced. Spores and capsules are not formed. It is gram negative, and stains readily with the ordinary aniline dyes. Various methods of isolation of the organism have been developed. It is not difficult to secure the organism in pure cultures from the

feces of Asiatic cholera patients by plating upon nutrient gelatin. The organism is aërobic and grows readily at room temperatures, although the optimum is blood heat. It is easily destroyed by high temperatures, 60° C. for a few minutes being sufficient. It is also easily destroyed by drying, by disinfectants, and by sunlight. It liquefies blood serum and gelatin. Milk is not coagulated. It grows best upon a medium which is neutral in reaction.

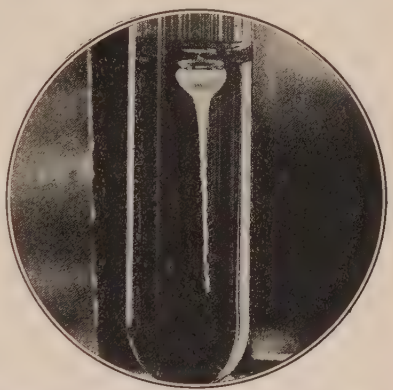


FIG. 172. — *Vibrio choleræ*, stab culture in gelatin showing liquefaction.

Disease Production. — The injection of cultures of the Asiatic cholera organism into laboratory animals in sufficient quantities usually proves fatal, but the disease produced is not typical Asiatic cholera. The disease as it occurs in man may be characterized as a severe type of diarrhea. The organism produces poisonous materials, possibly true toxins, which cause partial death or necrosis of a portion of the intestinal epithelium. This peals off, giving rise to the so-called rice water stools, the fragments of epithelium resembling particles of rice.

Immunity. — Agglutinins are developed in the blood of individuals having Asiatic cholera, but not early enough to be of great value in diagnosis. The blood of individuals that recover from the disease and that of animals that are artificially immunized contain bacteriolysins. It is possible that immunity to this disease is largely due to these substances. Vaccination has been extensively practiced, particularly in India. The vaccine consists of cultures that have been killed by exposure to a temperature of 58° C., or in some cases of cultures attenuated by growth at temperatures somewhat higher than their optimum.

Transmission. — This disease is generally transmitted through impure water and food. In Asiatic countries, as China, where night soil is commonly used in fertilization, the vegetables may

sometimes be infective. Bacillus carriers are of importance in this disease. During 1911 a number of cases of Asiatic cholera bacillus carriers were identified, by means of culture methods at the port of New York, among immigrants. Such individuals might easily serve as foci for an epidemic of the disease.

Non-pathogenic Spirilla

A considerable number of spirilla closely resembling the *Vibrio choleræ*, morphologically and culturally, have been isolated from water and other sources. These forms are worthy of note simply because they may cause confusion in the determination of the presence of the cholera spirillum in water. The only practicable method of differentiation in such cases is by the use of the blood serum of animals immunized against the various species which will give the specific agglutination reaction.

Spirochete Group

The organisms belonging to this group are elongated slender spiral forms resembling the spirilla among the bacteria. They are to be regarded as forms intermediate between bacteria and protozoa, partaking of the nature of both. The shape, lack of definite nucleus, and, according to some authors, the transverse division of the cells are true bacterial characters. Some authors have urged that multiplication usually takes place by longitudinal division of the cell. This is characteristic of many types of protozoa, though by no means of all of them. In some species undulating membranes differing from any membranes demonstrable in bacteria have been shown to be present. By certain staining methods granules have been demonstrated throughout the protoplasm. This is not unlike the scattered nuclei of certain of the protozoa. On the other hand, many bacteria show the same characters. Probably one of the best arguments for the protozoan relationships is the fact that some species seem to pass a part of their life history in the bodies of intermediate insect hosts such as ticks.

The only organism which will be discussed here is the *Treponema pallidum*, the cause of syphilis.

Treponema pallidum

Synonyms. — *Spirillum pallidum, Spirochæta pallida.*

This organism is the specific cause of syphilis in man. It was first described by Schaudinn and Hoffman in 1905. Before that date a considerable list of organisms had at one time or another been described as the cause of the disease, but these are known now to be secondary invaders entirely. The disease is one which is widely distributed among civilized and semicivilized peoples.

Morphology and Culture. — *Treponema pallidum* is an exceedingly slender organism less than 0.5 μ in diameter and usually about 4–20 μ in length. The spirals are relatively regular and may vary in number from three to forty or even more. The organism is actively motile. The organism was not recognized earlier because of the difficulty experienced in staining it. This difficulty also accounts for our lack of knowledge regarding many of its morphological characters. One of the simplest methods for demonstrating it is to mix the organism secured from infected tissue, particularly the initial lesion or chancre, with specially prepared India ink upon a glass slide and allow this to dry in a thin film. The organisms do not take up the ink and may be recognized after the film is dried as transparent spirals in a black film. When it is necessary to demonstrate them in tissues, complicated methods of staining are necessary.

The organism has been grown in pure cultures only very recently. Noguchi has worked out a satisfactory method for securing such cultures. A bit of fresh tissue taken from the liver, preferably of one of the laboratory animals, is dropped into a deep tube of liquid serum agar. A long platinum needle is infected with material containing *Treponema pallidum* and a long stab is made reaching to the bottom of the tube. The fresh tissues insure anaërobic conditions being maintained in the bottom of the tube. It is practically impossible to prevent the presence of organisms other than the Treponema in this culture. They are confined, however, largely to the line of stab, while near the bottom the Treponema grows out into the surrounding medium as an indefinite haze. If the surface of the tube is now sterilized and the tube carefully

broken, a pure culture of the Treponema may frequently be obtained from this hazy zone and inoculated into a tube similar to the one from which it was taken.

Disease Production. — The organism is readily found both in the primary and secondary lesions of syphilis and may sometimes be demonstrated in the tertiary lesions as well. The disease in a modified form may be transmitted to certain animals, particularly the rabbit and the anthropoid apes. The primary lesion in man is generally a chancre which develops in about three weeks after infection. The neighboring lymphatics are invaded, and there is more or less enlargement of the lymph nodes. About six weeks after the appearance of the primary lesion the secondary lesions occur. It is probable that these arise from the general invasion of the blood stream by the organisms. There is marked skin eruption, more or less falling of the hair, and usually fever. The duration of these symptoms is variable. Sometimes they last for years. A relative immunity may be established, but generally there are tertiary symptoms such as degeneration of the liver and changes in the blood vessel walls.

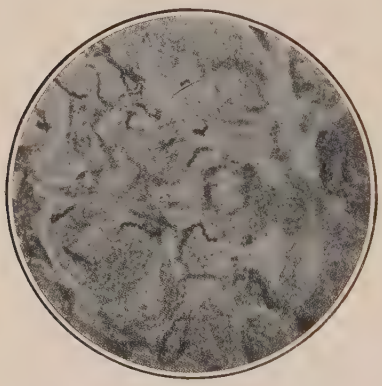


FIG. 173. — *Treponema pallida*, in a section of syphilitic liver.

Immunity. — No method of conferring immunity has been developed. Treatment involves the use of salts of mercury, iodine, and the organic arsenic compound arsphenamine. The disease is commonly diagnosed in the laboratory by means of the Wasserman complement fixation test.

Transmission. — Syphilis is primarily a sexual disease, usually transmitted by direct contact. Infection has been traced in some cases to infective drinking vessels. Although in the strictest sense it is not inherited, it may be transmitted from one generation to the next. Children of infected parents are often syphilitic at birth.

CHAPTER XLV

PATHOGENIC YEASTS AND MOLDS

Pathogenic Yeasts

Several species of organisms closely resembling the true yeasts morphologically have been described as the cause of certain specific diseases of man and animals. These organisms do not ferment sugars, and differ in other physiological and cultural characters

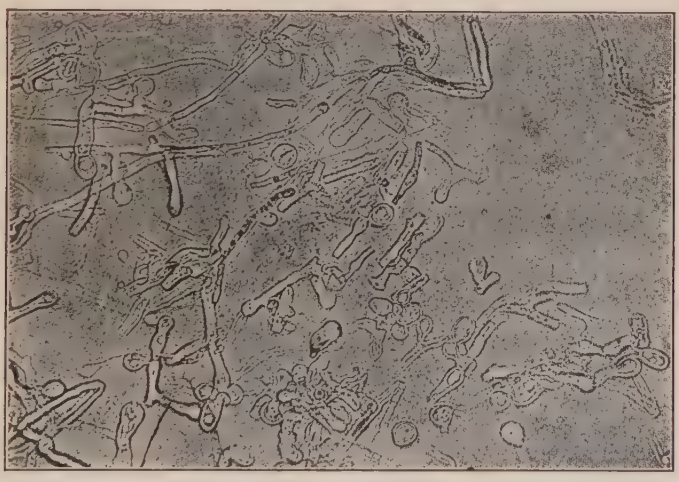


Fig. 174. — Blastomyces. (After Iron and Grahams, in *Journal of Infectious Diseases*.)

from the true yeasts. They have been given the genus name Blastomyces by some authors. They are characterized, as are the true yeasts, principally by their method of vegetative reproduction; that is, by budding. The diseases produced are comparatively rare and unusual. Two species have been recognized in the United States, *Blastomyces dermatitidis* and *Blastomyces coccidioides*. The disease caused by *Blastomyces dermatitidis* is generally mani-

fested by the development of papules upon the surface of the body from which a viscid pus is exuded. These finally heal with the appearance of a considerable amount of scar tissue. The disease progresses very slowly, usually the new ulcers appearing successively on various parts of the body. This disease has been noted principally from certain clinics in Chicago. *Blastomyces coccidioides* causes blastomycosis reported from portions of South America, and the western coast of the United States, particularly the San Joaquin Valley of California. This disease differs from the preceding in that the lesions are frequently not cutaneous, the infection being more generalized or systemic. It seems to be commonly fatal. Both of these diseases are relatively rare.

Pathogenic Molds

A considerable number of the Hyphomycetes or molds are known to produce disease in man and animals. In a majority of cases, the infections are more or less superficial, that is, of the skin and hair. The principal genera of molds containing species known to be pathogenic are Aspergillus, Microsporon, and Trichophyton, Achorion, and Oidium.

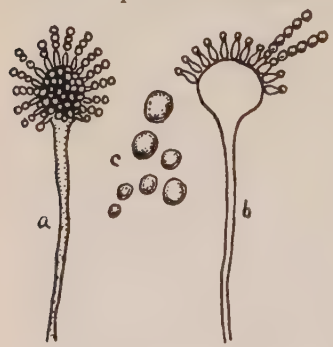


Fig. 175. — *Aspergillus fumigatus*. *a*, conidiophore with conidia; *b*, longitudinal section through conidial head showing the swollen tip of the conidiophore and the attachment of the conidial chains; *c*, conidia. (Adapted from Wehmer.)

The Genus Aspergillus

The characteristics of this genus have already been described in the chapter on molds. It is of importance from two points of view. First, when present in considerable numbers upon moldy grain and other feed, it may produce a fatal type of pneumonia in birds of many kinds, particularly barnyard fowls. The species involved is usually *Aspergillus fumigatus*. The organism produces its mycelium within the lungs and air sacs and bears its grayish or greenish spores upon conidiophores within the air passages. A

similar disease has been found to occur in man and animals, particularly the horse, when forced to breathe air containing the spores of this organism in great numbers. This and other species of Aspergillus are also important inasmuch as it seems very probable that they are capable of producing poisonous substances when growing in foods. Many instances of poisoning are known to have occurred among animals, due to the presence of these organisms in considerable numbers upon grain, hay, ensilage, etc. The exact nature of the poisonous substance produced has not been definitely determined.

The Genera Microsporon, Trichophyton, Achorion

These three genera constitute a group of organisms producing skin diseases of various types in man and animals.

Trichophyton tonsurans in one of its many forms is the cause of ringworm in man and many of the domestic animals. This disease is characterized by the formation of scales upon the skin and dropping out of the hair, with or without the formation of pus. It spreads slowly over the surface of the skin from the initial point of infection, hence the name of ringworm.

Achorion schoenleinii produces the disease favus or *tinea favosa* in man, various animals, and birds. This disease is characterized by scale formation upon the skin, usually with the accompaniment of disagreeable odors. These organisms may all be cultivated upon artificial media, and when so grown are found to belong among the simple types of the true Hyphomycetes.

The Genus Oidium

Oidium albicans produces thrush in children, sometimes in animals. This organism somewhat resembles the yeasts inasmuch as the cells multiply largely by budding and the mycelium is very poorly developed. The disease usually occurs in the mouths of sucklings. It is characterized by the appearance upon the surface of the mucous membrane of white patches, which vary in size from minute points to considerable areas. Infection is usually benign, but may extend to the pharynx or larynx. Occasionally the disease is fatal, owing to involvement of the internal organs.

CHAPTER XLVI

PATHOGENIC PROTOZOA

The protozoa hold the same relative position among animals that bacteria and fungi hold among plants. They pass their entire existence as single-celled individuals, although in some cases several or many cells may unite to form a colony. In no case, however, do the cells form a definite tissue or organ.

It has been noted in a previous chapter that there are many intergradations between the protozoa and bacteria. The genus Spirochæta is variously regarded. By some authors it is grouped with the bacteria, by others with the protozoa, while others believe (and probably this view is justifiable) that it is a form intermediate between the two groups, partaking in part of protozoan and in part of bacterial characteristics. Our discussion of protozoa must be limited to the comparatively small number of genera and species which are known to produce disease in man. Many hundreds and even thousands of species are known living in the bodies of other animals. The genera to be considered are Amœba and Entamœba, Trypanosoma, and Plasmodium.

The Genera Amœba and Entamœba

The organisms of these genera belong to the class *Sarcodina* (Rhizopoda) of the protozoa. This group includes those protozoa which during their adult life have moveable or changeable protoplasmic processes termed pseudopodia. A definite membrane is lacking, the protoplasm moving about by a kind of flowing motion. They reproduce by fission and by the formation of spores. Several hundred genera with numerous species have been described belonging to this group. One quite certainly, and probably two or three, are known to be pathogenic for man. The *Entamœba coli* is regarded as a more or less normal inhabitant

of the intestinal tract in man, and somewhat similar species have been noted from the intestinal tracts of other animals.

It is possible to cultivate many species of amœbæ upon artificial media. The amœbæ differ from organisms previously studied in that they utilize living cells as food. These cells are engulfed by the protoplasm which secretes a digestive enzyme, and the food particles then lie in a digestive vacuole or temporary stomach. It is essential, therefore, if amœbæ are to be cultivated, that they be supplied with living cells for food. This may be accomplished by growing bacteria upon the surface of a medium containing only sufficient nutrients for moderate development. Amœbæ can move about over the surface of this medium and engulf organisms as needed for food.

Entamœba histolytica

Entamœba histolytica (*Amœba dysenteriæ*) is the specific cause of amœbic dysentery in man. The organism may be found in stools by mixing a small quantity of the material with physiological salt solution and placing this upon a slide under a cover glass. The slide must be kept warm if motion is to be observed. The organism is usually actively motile, throwing out pseudopodia on all sides. It appears as a clear mass of protoplasm, sometimes tinged with green from the hemoglobin of the blood that has been ingested. It is not difficult to differentiate, in the larger cells at least, the two layers of protoplasm, the so-called endoplasm or inner portion, and the ectoplasm or covering. The ectoplasm is glasslike and refractive. The pseudopodia which are thrown out, and by means of which the organism moves, consist largely of ectoplasm. The endoplasm in active cells usually contains a considerable number of vacuoles. The amœba reproduces in two ways, by the vegetative type of multiplication common to all amœbæ, and by sporulation.

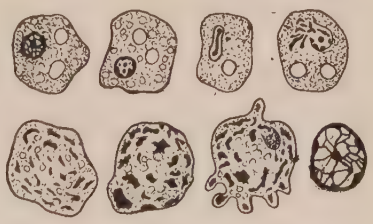


Fig. 176. — *Entamœba histolytica*, various stages in the development of spores. (Adapted from Craig.)

The spores of *E. histolytica* fed to kittens produce typical amœbic dysentery with characteristic ulcerations of the intestinal wall. The disease in man is a chronic or persistent dysentery, usually accompanied by considerable ulceration of the intestines and not infrequently by abscesses in the liver. No method of immunization against this organism has been developed. It is a disease transmitted by impure water, possibly by contaminated food. It is more common in tropical than in temperate countries.

The Genus Trypanosoma

The trypanosomes are parasites which have been found to occur in the blood of many species of animals, fish, reptiles, and birds, in some cases seemingly producing no injurious effects, in other cases causing highly fatal diseases. The trypanosomal cell is elongated, usually more or less spindle-shaped, tapers more or less to the ends, and is several times as long as broad. The anterior end is usually tipped with a flagellum, and longitudinally along one side of the cell is a thin, undulating membrane. The flagellum originates in the anterior portion of the cell near a deeply staining granule, extends along the outer edge of the membrane, and is only free at the very tip. The flagellum, therefore, is in part within the protoplasm of the cell, for a portion of its length attached to the edge of the undulating membrane, and usually free in part. Usually the organism is actively motile. The individual cells can vary their shape to a considerable degree. Multiplication occurs through longitudinal splitting of the cell. It is probable that some species of trypanosomes undergo a series of changes or a developmental cycle in the body of certain intermediate hosts.

Fig. 177. — Trypanosome. *a*, nucleus; *b*, undulating membrane; *c*, flagellum. (Adapted from Gonder and Sieber.)

Many species of trypanosomes may be cultivated in the laboratory. The method was first described in 1903 by Novy and MacNeal, who mixed equal portions of defibrinated rabbit blood and melted nutrient agar. This was allowed to solidify, slanted, and the trypanosomes inoculated into the water of condensation.

By transferring from one tube to another they successfully cultivated the organism for a considerable period of time.

Many species of trypanosomes, as has already been noted, appear in animals without seriously interfering with health. It has been found, for example, that it is not difficult to cultivate a trypanosome from the blood of cattle, although it has never been demonstrated except in cultures. The rat frequently harbors trypanosomes in the blood. Birds, fish, and reptiles often have in their blood trypanosomes or representatives of closely related genera. Many diseases of animals, such as surra in horses and cattle, the nagana or tsetse fly disease in horses, cattle, camels, etc., and a considerable list of similar diseases affecting domestic and wild animals, have been described.

Trypanosoma gambiense

Synonyms. — *Tr. ugandense, Tr. castellani.*

This is the cause of human trypanosomiasis or sleeping sickness. The disease has been known among the negroes on the western coast of Africa for more than a century. Within recent years it has spread to a marked degree, and some areas of Central Africa have been completely depopulated and rendered uninhabitable. It is the principal foe to the development and settlement of large areas in that country. At the present time it is spreading slowly, but vigorous methods are being taken to control it.

FIG. 178. — *Trypanosoma gambiense* of sleeping sickness. (Adapted from Nabarro.)

Morphology. — The organism is 1.4 μ — 2 μ × 17 μ — 28 μ. The free flagellum may be from one-fourth to one-third the length of the body. The undulating membrane is relatively narrow.

Disease Production. — The disease may be produced by the injection of infected blood into the monkey, dogs, cats, and various other laboratory animals. The disease in man is insidious in its onset. At first the organism appears in the blood and there usually is more or less fever. In the second stage pains develop in the back. The patient becomes exceedingly drowsy and can be

aroused only with difficulty. Finally he settles into a state of coma and dies. The organism may be demonstrated in this second stage in the cerebrospinal fluid. The disease is probably always fatal, but may run a somewhat chronic course. No method of immunization has been developed.

Transmission. — One of the bloodsucking flies, the so-called tsetse fly (*Glossina palpalis*), has been found to transmit the disease. It has been shown that the fly after it has fed upon the blood of a patient having the disease may infect laboratory animals within the next two days when allowed to feed upon them. It then loses its power of infection, but regains it in about three weeks. It seems probable therefore that the organism passes through certain developmental stages within the body of the fly, and after these changes the insect again becomes infective. Some effort is being made to control the disease by destruction of the breeding places of these flies along water courses and to prevent these insects from biting infected individuals.

The Genus Plasmodium

Three species of protozoa, commonly included in the genus Plasmodium, have been determined to be the specific causes of three types of malaria in man. In every case the organism goes through certain changes (a developmental cycle) in the red blood cells of man, and the remainder of the life cycle is carried out in the digestive tract and tissues of the mosquito. Plasmodium was first described by Laveran in 1880, and since that time the three types have been differentiated. The first of these is

Plasmodium vivax

This is the cause of the disease of man known as tertian malaria. This is the common malaria or " ague " of temperate climates.

Morphology and Life History. — The organism when it first enters the human blood is small and amœboid. It soon penetrates a red blood cell, enlarges and develops in the interior of the cell until this is filled. It then segments to form a rosette or small spores or merozoïtes. These escape from the red blood corpuscle, each one attaches itself to another cell, penetrates it, and begins

development anew. This growth to full size followed by sporulation and infection of new cells may be repeated several times. It is termed the asexual phase in the life history of the organism. The disease is always characterized by chills and fever which develop at the time when the organisms escape from the corpuscles and infect others. It is possible that certain poisonous materials are liberated at this time. In order to complete the life cycle, the organism must pass into the body of the mosquito. This insect takes it up with blood. In the gut of the mosquito two types of cells are formed, corresponding to eggs and sperms. Each egg is fertilized by a sperm and burrows into the stomach wall. Here it becomes considerably enlarged and breaks up into a number of spherical cells called sporoblasts. The contents of these in turn are transformed into large numbers of delicate filamentous cells termed sporozoïtes. The mother cell wall ruptures and the sporozoïtes pass into the body cavity of the mosquito. From here they make their way to the poison or salivary glands and pass into the blood of the individual next bitten by the insect. It is evident that during the sexual cycle of the organism, the insect remains non-infective. From eight to ten days usually elapse between the time the blood is taken into the body of the mosquito and the appearance of the organism in the poison glands. The malaria produced by this type of Plasmodium is usually benign, rarely terminating fatally. The chills and fever appear every two days, as this is the time required for the organism to pass through its asexual cycle in the human blood.

No method of immunization has been developed. Malaria, as was emphasized above, is transmitted to man only by the bite of an infected mosquito. Prevention of the disease necessitates the destruction of the breeding places of mosquitoes, and when such bodies of water cannot be drained, they must be oiled to kill the larvæ.[1] Care must likewise be used to see that the mosquito does not bite malarial patients and be thus rendered infective. Lastly mosquitoes should be prevented from biting healthy individuals.

[1] The mosquito passes the first stages of its life in the water; only the mature insect is a bloodsucker.

Plasmodium malariæ

This organism produces the so-called quartan malaria in which the interval between the paroxyms of fever is seventy-two hours. The disease is relatively benign, and is transmitted in the same manner as the preceding.

Plasmodium immaculatum and P. falciparum

The malarias produced by these organisms are malignant and do not yield readily to quinine treatment. Two types are known, a quotidian in which the asexual cycle is complete in twenty-four hours, and a tertian in which it is complete in forty-eight hours. These are transmitted in the same manner as are the preceding types. They are the most usual malarias of the tropics.

CHAPTER XLVII

DISEASES WHOSE CAUSES ARE NOT KNOWN

There is a considerable number of diseases whose causes at the present time are unknown, uncertain, or belong to the group of ultramicroscopic or filterable viruses. It is impracticable among the last to tell exactly whether we are dealing with protozoa or bacteria or neither. In some cases growth can be secured in a culture medium; in such cases it is probable that the organisms are bacteria. In most cases it is impossible to classify them certainly.

Virus of Smallpox

The organism which produces smallpox is not certainly known. Inclusions of the cells in the pustules characteristic of the disease have been described as probably protozoan in nature, but this has not been definitely demonstrated. The protozoön, if such it be, has been named *Cytorrhectes vacciniæ*. The organism, at least at some stages in its history, can pass through the pores of a coarse porcelain filter and is probably ultramicroscopic. It has never been cultivated upon artificial media.

Immunity. — An attack of smallpox confers a relatively lasting immunity upon the individual. For several centuries at least vaccination against smallpox has been practiced. Originally it consisted of the inoculation into a healthy individual of the smallpox virus obtained from a pustule of a mild case of the disease. It was noted by Jenner in 1796 that milkmaids who contracted the disease cowpox from milking infected cows were relatively immune thereafter to smallpox. It seems probable that both diseases, smallpox and cowpox, are caused either by the same, or by very closely related, organisms. At any rate, vaccination

against smallpox with material taken from cowpox pustules is commonly practiced at the present time. The vaccine now used is prepared with great care. Young heifers are usually chosen for its preparation. They are examined carefully to ascertain that they are entirely free from any infectious disease. They are carefully washed and kept in stables that can be thoroughly cleansed. The skin covering the abdomen is shaved and cleaned thoroughly. It is then scarified by means of a curette, parallel lines being made over the greater portion of this area. Lymph obtained from a previously vaccinated animal is then rubbed into this scarified area. Within several days (usually from five to seven) more or less inflammation will have developed in the skin and subcutaneous tissues, together with characteristic vesicles containing lymph. The contents of these vesicles are carefully removed and mixed with 50 per cent glycerol. Tests are made by inoculation into guinea pigs to determine that there are no pathogenic organisms other than smallpox virus present. Particular care is used to exclude *Clostridium tetani*. This lymph is prepared for use by drawing it into capillary pipettes or by allowing it to dry on sterile ivory or bone points. The organism retains its vitality under these conditions for considerable periods of time, and when rubbed into scarified skin, produces an infection termed *vaccinia*. In some cases the material used originally in producing pustules on the bodies of the calves came from cases of smallpox. It is not improbable that cowpox is therefore to be considered as a much attenuated form of smallpox, or that the smallpox virus when injected into the body of cattle in large measure loses its virulence for man. The infection by scarification when properly performed rarely leads to serious results, and the individual becomes thereby relatively immune to smallpox. Vaccination is to be regarded as infection with an organism so attenuated that the disease will run a benign course.

Virus of Chicken Pox

The organism responsible for the production of chicken pox has not been determined. It doubtless is related to the one which causes smallpox, and like it is probably filterable.

Virus of Yellow Fever

The disease yellow fever is one which usually is confined to tropical countries, but occasionally during warm seasons invades more temperate climates. It has been definitely shown to be due to a filterable virus, probably to an ultramicroscopic organism that has never been cultivated. In some respects, yellow fever resembles an exceedingly virulent type of malaria, and like malaria it is transmitted by the bite of the mosquito and in no other manner. The mosquito responsible for the spread of the disease is *Stegomyia fasciata*. It is probable that, like the malarias, the virus of yellow fever passes through part of its life cycle in the body of the mosquito, for this insect does not become infective and cannot transmit the disease to another individual for a number of days after taking infected blood into its alimentary tract. The mosquito evidently serves as a true intermediate host.

No practicable methods of immunization against yellow fever have been developed. The disease may be stamped out by protecting yellow fever patients from the bites of mosquitoes and by preventing as far as possible mosquitoes from biting uninfected individuals. Most important, probably, is the elimination of the breeding places of these mosquitoes. The recognition of the means by which this disease spreads has enabled the sanitarian to eradicate the disease from such cities as Havana in Cuba, where it has been known to be constantly present for a large part of the past century. This knowledge also renders very improbable any extensive invasion of our Southern states, as has happened in the past.

Virus of Typhus Fever

Typhus fever is classed among the exanthemata or eruptive fevers. It is not to be confused with typhoid fever, which is an entirely distinct disease, although the two were not differentiated with certainty until the middle of the last century. Typhus fever is not uncommon in certain parts of Mexico and other tropical countries. It was once common in the slums of the larger cities of Great Britain and Europe, and has recently been shown

to exist in attenuated form in some of the cities of the United States. The organism is probably an ultramicroscopic virus. The disease is transmitted by lice, particularly the body louse (*Pediculus vestimenti*), and possibly by the head louse (*P. capitis*) as well. Methods of immunization have not been developed.

Virus of Rocky Mountain Spotted Fever

Spotted fever or Rocky Mountain spotted fever is a disease which was first noted because of its virulence in the Bitter Root Valley of Montana. It is now known to occur in a large portion of the area between the Cascade Mountains of Oregon and Washington and the central portions of Montana and Wyoming. In most districts from which it has been reported it is relatively mild, the mortality being low. In the Bitter Root Valley, however, the virus seems to be particularly virulent and the mortality of those infected is high. Ricketts and his co-workers have shown that the disease is transmitted by the bite of certain ticks. It is probable that rodents, possibly also other animals of the infected region, harbor the organisms or may be infected with the disease. In the laboratory it has been found to be transmissible to the guinea pig and some other rodents. Ticks biting these animals, and later biting man, may transmit the disease to the latter.

It is probable that the causal organism in this case is a tiny pleomorphic gram-negative form to which the generic name Rickettsia has been given. Whether this is to be regarded as a bacterium or a protozoan is uncertain, but probably the latter.

Virus of Epidemic Infantile Paralysis

This disease, also known as acute poliomyelitis and Heine-Medin disease, has been described within recent years in several European countries and in the United States. The causal organism was shown by Flexner and Lewis in 1909 to be a filterable virus, and in all probability ultramicroscopic. The disease is one which affects primarily the spinal cord. It has been developed in typical form in the monkey by suitable injections. It is believed that it is spread by ingestion of infective materials. Methods of

treatment and prevention by means of antisera have not been conclusively tested.

The Virus of Rabies or Hydrophobia

The disease known as hydrophobia in man, and rabies or lyssa in animals, is probably caused by a protozoan parasite which has been described by Negri as *Neuroryctes hydrophobiæ*. It is claimed by several investigators that the virus may be passed through a porcelain filter, so that in certain stages at least, it is ultramicroscopic. Negri, using stained sections showing the larger ganglion cells of the Ammon's horn of the brain, demonstrated the presence of specific bodies which have since been termed Negri bodies. There seems to be little question but that these organisms are quite constantly present in diseased animals and are not present in animals that do not have the disease, but it is not quite so certain whether they are specific organisms or are simply degeneration products. These Negri bodies in suitably stained preparations are found to vary in size from 0.5 to 25 μ in diameter. They may be ovoid, ellipsoidal, or spherical. They generally show minute granular portions with inclusions of various kinds resembling chromatin granules.

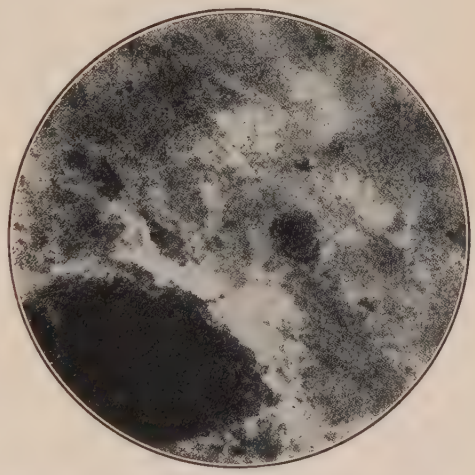


Fig. 179. — Negri bodies. (After Williams and Lowden.)

The disease seems to be primarily one of dogs and related carnivora. It usually is transmitted to man by the bite of a diseased animal. It has been shown that the saliva of such animals is infective. The organism thus enters the body through wounds

and passes along the peripheral nerves to the central nervous system. The portion of the central nervous system to which these nerves lead directly is in consequence the one usually most seriously affected. Some little time elapses between infection and the development of the symptoms of the disease. The period of incubation is quite variable, but is frequently several weeks. The disease is fatal in most cases.

The method of vaccination for rabies was developed by Pasteur. He found that the specific virus present in the spinal cord and brain of infected dogs shows considerable variation in virulence. His fixed virus is produced by repeated inoculations of rabbits until the virulence is sufficiently exalted so that the injections will kill these animals in from six to seven days. A rabbit is then injected, and upon its death the spinal cord is carefully removed, using all aseptic precautions. This is then suspended in a desiccator over caustic potash and kept at a constant temperature of 23° C. in the dark for a week. The vaccine is prepared by emulsifying this cord. This drying process seems to attenuate the organism so that injections of the cord may be made without danger to the individual who has been injected, at the same time conferring a certain amount of immunity. Later other injections are made from cords that have been dried for shorter periods of time. The long incubation period of the disease gives an opportunity for repeated injections of material of this kind so that a considerable degree of immunity is established before the disease has had an opportunity to manifest itself.

Virus of Influenza

The disease influenza is an acute infection of man which has swept in great pandemic around the world in recent years. The ordinary form shows an onset of severe headache accompanied by pains and aches in the back, by fever and by general prostration. The fever continues in such cases from 3 to 5 days and finally leaves the patient exhausted.

Pfeiffer in 1892 described an organism which he believed to be the cause of the disease influenza. This has been generally assumed to be the cause until investigation in the past few years

failed to prove any causal relationship between this organism and the disease. While much work has been done upon the disease, the causal organism has not yet been isolated with certainty. More recently it has been claimed to be due to a streptococcus.

The disease is important not only in itself but because it is followed in a considerable proportion of cases by severe and frequently fatal pneumonia.

CHAPTER XLVIII

WATER CONTAMINATION, EXAMINATION, AND PURIFICATION

The Flora of Relatively Pure Water. Normal Water Bacteria

Sterile water is rarely found in nature. Water obtained even from deep wells and springs usually contains a few bacteria. Surface waters, those of streams, ponds, lakes, and the water of the ocean, always contain bacteria in greater or smaller numbers. These organisms may be designated as the normal water flora as they are constantly present and in no case are capable of producing disease in man. For the most part, they are organisms that grow best at the temperature of the water in which they are found.

Cocci are quite common in water. They are largely chromogenes, either micrococci or sarcinæ. Probably the commonest of these is *Sarcina lutea*, a form developing lemon-yellow colonies when grown upon artificial media. *Sarcina aurantiaca*, which produces a golden yellow pigment, is also common, as are other cocci producing pink, red, yellow, and orange pigments. Certain non-chromogenic cocci, as *Micrococcus candicans*, may also be found.

Chromogenic bacilli such as *Serratia marcescens* (red pigment), *Flavobacterium aurantiacum* (orange pigment), and *Chromobacterium violaceum* (violet pigment) are common in surface waters. Bacilli such as *Pseudomonas fluorescens*, which form fluorescent colonies upon media, are also commonly present. Whenever water receives a considerable amount of surface drainage, the normal soil bacteria such as various members of the *Bacillus subtilis* group are practically always present.

A pure water may be defined as one which contains no disease-producing bacteria nor an excess of organic matter of any kind. The presence of considerable quantities of organic matter leads to the multiplication of many putrefactive bacteria, such as the spore-bearing anaërobes. While these are not necessarily injurious to health, they are undesirable.

Flora of Impure Water

The organisms of importance in impure water may be either pathogenic or non-pathogenic. Among the pathogenic micro-organisms which may gain entrance to water supplies through contamination with sewage are *Bacterium typhosum*, *Bact. dysenteriæ*, *Vibrio choleræ*, and certain other related organisms producing diarrheas and dysenteries.

An impure water is generally defined as one which receives more or less sewage, one therefore that is not fit for human consumption because of the possible presence of pathogenic bacteria. Relatively few species of organisms are held to be indicative of sewage pollution. The most important of these are *Bact. coli*, *Bact. aërogenes*, *Streptococcus pyogenes*, and the various types of the proteus group. These organisms have already been described and their constant presence in the intestines of man and animals noted. When they are found to occur in any considerable numbers in water, it is to be concluded that such water is contaminated with sewage inasmuch as these forms do not continue to multiply outside of the body for very long periods after their elimination from the body.

Relationship of Water to Health

A pure water supply is one of the best safeguards of the health of a community. A relatively small number of diseases, however, are actually transmitted by impure water. All of these are diseases in which the infection atrium is the alimentary tract and in which the organisms leave the body with the excretions. The most important of these diseases are typhoid fever, dysentery, and Asiatic cholera.

A pure water supply, however, seems to affect the general health of a community and to decrease its death rate wholly apart from the decrease due to the elimination of these specific diseases mentioned. It has been noted, for example, that when a city changes from a contaminated to a pure supply, there is not only a marked decrease in the typhoid death rate, but also in some instances decreases are noted in the number of deaths from tuberculosis, pneumonia, and other diseases. In practically every instance there is a decided decrease in the total death rate. This has been termed the Mills-Reincke phenomenon, after the men who first called attention to these facts. This phenomenon must be due to one of two causes. Either diseases not ordinarily regarded as being carried by water are in a certain percentage of cases so transmitted, or the use of an impure water supply so affects the average health of a community that the individuals become more readily susceptible to other diseases. It is probable that the latter is the true explanation.

Bacteriological Examination of Water

The methods used by the bacteriologist in making an examination of water to determine whether or not it is potable may be differentiated into quantitative methods and qualitative methods. In the former, the total number of bacteria present is determined. In the second, an effort is made to determine the kinds of bacteria. Both of these methods are useful, and they are generally employed together.

A sample of water for bacteriological analysis must be collected carefully. It is customary to use wide-mouthed, sterile, glass-stoppered bottles. If the sample is to be taken from a well, sufficient water must be pumped to remove that which has been standing in the pipes in order that a sample representative of the water in the well itself may be secured. If the collection is from a tap, the water should be allowed to run for a time before sampling. If it is taken from a stream or body of water, the unopened glass bottle should be thrust below the surface before removing the stopper, the bottle filled below the surface, and the stopper returned. This avoids collecting the dust which may be present

upon the surface and thus vitiating the results. When samples are taken at a depth in a body of water, various devices are used whereby a bottle or flask is lowered and automatically opened and closed at the depth required. When the sample has been collected, it should be taken to the laboratory and examined at once. If it is necessary to send the water for some distance, it must be packed in ice and hurried to its destination as quickly as possible. These precautions must be taken, as the conditions under which the water is held in the bottle are quite different from those under which it was secured. If the water comes from a cold spring, for example, the bacteria present begin to multiply rapidly. Within a few hours they may be hundreds or even thousands of times as numerous as in the original sample, and an examination of such water will not reveal the true condition of the original supply at all. The more highly contaminated the water, the more essential is a prompt examination.

Quantitative Methods of Water Examination

The number of bacteria present in a water sample is determined by pouring gelatin on agar plates. Various dilutions of the water to be examined are prepared by means of water blanks of known volume and sterile pipettes.[1] One cc. of each of the dilutions to be examined is placed in a sterile petri dish and a tube of sterile gelatin or agar poured into this, thoroughly mixed with it by tilting, and allowed to stand in a cool place until the medium has hardened. This effectually prevents the bacteria from moving about, and colonies develop from these in the medium. It is customary in making an analysis of this kind to hold the plates at about 20 or 22° C., as most of the water forms develop well at this temperature. In most cases the number of colonies on these plates can be counted at the end of forty-eight hours. The number of colonies that have developed on a plate multiplied by its dilution should give the approximate number of organsims per cubic centimeter pres-

[1] A water blank is a test tube or flask containing a definite volume of usually 9 cc., 49 cc., 95 cc., or 995 cc. of sterile water. By the addition of 1 cc. of water, for example, to 9 cc. of sterile water, a dilution of 1–10 is effected. Other dilutions are prepared in a similar manner. The pipettes used usually have a capacity of 1 cc. or 5 cc.

ent in the original sample. This, however, is true only within certain limits. When bacteria are too closely crowded on the plate, the growth of one colony may inhibit the development of others. It is customary, therefore, to count that dilution which shows no more than 200 bacteria on the plate, because the presence of a larger number will ordinarily give too low a total count after multiplying by the dilution. If it is desired to identify the organisms present, representatives of each type of colony may be transferred to other artificial media and the cultural, physiological, and morphological characters ascertained.

It is not always easy to interpret the results of a quantitative analysis of water. It is necessary first that we know something of the number of bacteria to be expected in a sample of water that is not polluted. In general, it is true that pure waters contain small numbers of bacteria and polluted waters contain large numbers. Water from a deep well or spring may sometimes be obtained which is wholly free from microörganisms or at the most contains no more than 10 or 20 organisms per cubic centimeter. The water in ponds and lakes is rarely as pure as this, but when there is not an excess of organic material and no sewage pollution, the number rarely runs above 200 or 300. The same may be true of pure stream waters except during seasons of high water, when considerable amounts of surface material containing soil organisms may be washed into the stream. A polluted water is one which receives a considerable amount of sewage. It usually contains several thousands, sometimes even millions, of organisms per cubic centimeter. In general it may be stated that water containing less than 100 bacteria per cubic centimeter is probably pure, while water containing 500 per cubic centimeter is to be regarded as suspicious, and one containing more than 1000 organisms per cubic centimeter is bad. These results taken alone, however, do not mean much. In a final determination of potability, it is usually best to make qualitative examinations as well. In some cases, however, examinations of water by quantitative methods have led to valuable results. In the larger water purification plants where the water passes through sand filters, it is customary in many instances to determine the extent of the purification

of the water by an analysis at intervals as it leaves the filter beds. A filter when working efficiently should remove a very high percentage of the bacteria present in the original supply. It is therefore possible relatively quickly to recognize defective action of a filter.

Qualitative Methods of Water Examination

Qualitative water examinations are made for one of two purposes, either the isolation of specific pathogenic organisms or for the recognition of typical sewage bacteria.

Isolation of Specific Pathogens. — The isolation of specific pathogenic organisms such as *Bacterium typhosum* or *Vibrio choleræ* from a contaminated water supply is difficult. This is due to several causes. Although pathogenic bacteria may be present in a water supply, it is not often that they are constantly present. The attention of a city, for example, is not called to a defect in its water supply until the outbreak of an epidemic of typhoid fever, and since the incubation period of typhoid fever is usually from nine to ten days, before examinations are begun upon such a water supply, the organisms have in all probability disappeared from it. Even when constantly present, they usually are relatively scarce. Even in the stools of a typhoid patient the cells of *Bact. coli* outnumbers the *Bact. typhosum*. When cultivation is attempted upon artificial media, therefore, the typhoid bacteria are usually overgrown. Successful isolation from water supplies has been effected in only a few instances. In these cases water has been inoculated into solutions which favor the growth of *Bact. typhosum* (and other intestinal organisms) at the expense of other bacteria. Then by means of plate cultures the typhoid and the colon bacilli have been differentiated. Isolation of a specific pathogen, however, is but rarely attempted, and is of scientific interest only.

The sewage bacteria for which search is usually made are the *Escherichia* (*Bacterium*) *coli:* (and closely related *Aërobacter* (*Bacterium*) *aërogenes*) and streptococci. In England the spore-bearing anaërobe *Clostridium enteritidis sporogenes* (probably a synonym of *Clostridium welchii*) is sometimes sought. The meth-

ods used may be divided into two general groups, the presumptive methods and those in which definite identification of *Bact. coli* is made.

Presumptive Methods. — Advantage is taken of certain of the physiological characters of *Bact. coli* to differentiate it from other organisms. The media commonly used are dextrose broth and lactose bile in fermentation tubes. When various amounts of

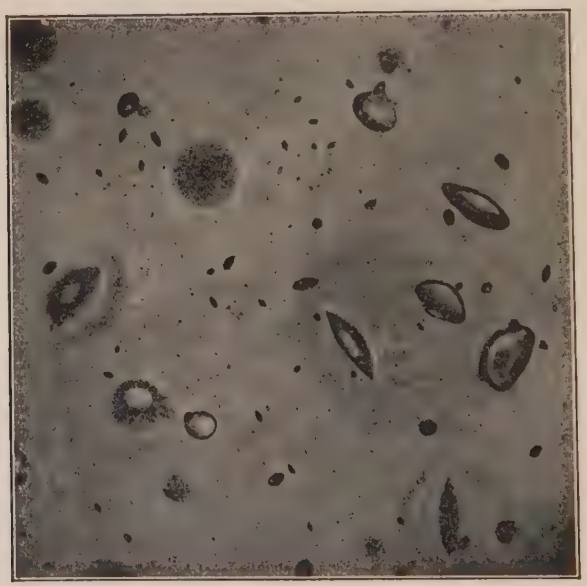


FIG. 180. — Bacterial colonies and gas bubbles formed in litmus lactose agar plates poured from sewage. (× 20.)

water to be examined are inoculated into fermentation tubes containing dextrose broth kept at blood heat for twenty-four hours, gas will be produced in those tubes in which *Bact. coli* is present. It is probably safe to infer that *Bact. coli* is not present if the gas is not produced in any of the tubes inoculated. The reverse, however, that *Bact. coli* must necessarily be present whenever gas is produced, does not hold good, for there are other organisms which can ferment dextrose with the production of acid and gas. The test is most reliable when a two per cent solution of lactose

bile is substituted for the dextrose broth in tubes. The bile inhibits the growth of most organisms not of intestinal origin. Most of the forms other than *Bact. coli* cannot form gas from lactose. Gas production, under these conditions, therefore, in the great majority of cases means the presence of *Bact. coli.* The absence of gas production indicates that this organism is not present. These tests are termed presumptive tests, because while they enable one to recognize waters which are suspicious, they do not certainly identify the organism responsible for the gas in the suspicious cases. This enables one, in other words, to divide waters tested into two groups, those which are certainly good and those which are suspicious. The latter require further examination.

Identification of Bacterium coli. — A considerable number of methods have been devised for the differentiation of *Bact. coli* either directly from water or from the dextrose or lactose bile tubes discussed above. When water containing *Bact. coli* is plated on agar containing one per cent lactose and sufficient litmus solution to color it blue, then incubated for twenty-four hours at blood heat, the lactose-fermenting colonies will be found to be surrounded by a red zone.

More frequently use is made of Endo agar or eosin methylene blue in confirming the results of positive presumptive fermentation tests noted above. Endo medium is lactose nutrient agar containing the dye basic fuchsin which has been decolorized by the addition of sodium sulphite. A loopful of broth from a fermentation tube showing gas production may be spread over the surface of the medium. Colonies of *Bact. coli* are characterized by the restoration of the red color in the medium, and the development of a green sheen. The eosin-methylene blue medium is also a lactose nutrient agar to which suitable amounts of these dyes have been added. *Bact. coli* grows well, and develops a dark colony with a green sheen; *Bact. aërogenes* likewise develops well with the formation of colonies without the metallic sheen. These media are used extensively, as the presence of *Bact. aërogenes* does not have the same sanitary significance as the presence of *Bact. coli.*

Interpretation of Results. — It is probable that *Bact. coli* may actually gain entrance to water in small numbers without neces-

sarily indicating serious sewage contamination. Water wholly free from *Bact. coli* is certainly above suspicion. A standard commonly accepted is that water containing less than one organism of *Bact. coli* per 50 cc. is safe for use, while water containing more than that number is probably contaminated with sewage and should not be used. It should be emphasized that the reason for avoiding water containing *Bact. coli* is not that this organism itself is necessarily harmful when taken into the body, but because of the fact that it indicates sewage pollution and the probable presence in such a water sooner or later of pathogenic bacteria from the intestines.

Purification of Water

Water at rest in ponds or lakes or running in streams tends to purify itself. This is due to a variety of factors. The organisms present in impure water tend to settle to the bottom, or undergo *sedimentation*. This is due in part to the phenomenon of adhesion. Bacteria tend to collect in clumps or to attach themselves to the surface of solid particles in the water and are carried to the bottom with these particles. Once at the bottom, conditions are usually so unfavorable for continued development that death soon occurs. This is particularly true of the pathogenic forms that may be present. The water of any flowing stream also usually undergoes a certain amount of *filtration*. A portion of the water flows through the sand in the bed of the stream; water plants and algæ tend to retard the current and to catch microörganisms which may be present. It is probable that *light* may sometimes destroy the bacteria in water, but its effect is not felt at any distance from the surface, and in most cases it is not of primary importance. Microörganisms characteristic of sewage, particularly the pathogenic forms, generally find the temperature and food conditions in water not suitable for continued growth. In addition, when water is highly polluted, the putrefactive and decay-producing bacteria develop so rapidly that the pathogenic forms are destroyed as a result of *antibiosis*. Many species of *protozoa* live in polluted water, their food consisting of bacteria and related microörganisms. These protozoa are prob-

ably of considerable importance in reducing the numbers of bacteria in some instances.

Disposal of Sewage. — Sewage is water containing a considerable proportion of household waste of different kinds and excretions of the body. Care should be used in most instances in its disposition for two reasons, first because when allowed to stand in pools it may develop odors and create a nuisance, and second it may pollute water supplies. Many methods of disposal of sewage have been advocated, most of them successful under certain conditions, but none of them seemingly applicable to all conditions. The easiest method of sewage disposal and one which is quite generally used is to allow it to run into a stream, lake, or some other body of water. If the stream is of sufficient size so that there is not such a concentration of sewage as to create a nuisance and the water is not used for domestic purposes, there seems to be no objection to this method of disposal. The same is true where sewage is allowed to run into the ocean or some arm of the ocean where it is continually washed away by the action of the tide and greatly diluted, or where the sewage empties into a lake. There are many cases where cities use a lake both for the disposal of sewage and as a source of drinking water, and other instances where one city uses a river bed as a sewer and another as a source of water supply. In such cases other methods of sewage disposal should be sought. The disposal of sewage of inland towns which are not situated on the banks of streams of considerable size is attended with difficulty. Many of the towns of the Middle and Western states of the United States, for example, dispose of their sewage by allowing it to run into some water channel which ordinarily would be wholly dry during a portion of the year. Sewage under these conditions stagnates, and creates a nuisance by development of unpleasant odors.

The solid material in suspension and part of that in solution may be removed from sewage by the use of coagulants. This method of disposal has been extensively used in England. The sewage is allowed to run into large vats where alum, lime, sulphate of iron, or some similar compound is added and thoroughly mixed. This causes a coagulation and sedimentation of the solid material.

The clear sewage is then allowed to run into a stream. A sufficient quantity of organic matter is usually removed to insure the absence of putrefaction and the development of obnoxious odors. The precipitated material or sludge may be burned or utilized as fertilizer.

Some of the large cities of Europe, such as Berlin and Paris, and some of the cities on the western coast of the United States dispose of their sewage by means of irrigation sewage farms. This can be economically carried out where large areas of sandy land are available and where the climate is dry. With a heavy soil and moist climate, however, it is not very practicable. The sewage is spread out over the land as is customary in irrigation, and the excess is carried off by a system of underground tiles. Crops are grown upon these sewage farms and are a source of considerable income to those cities where this system is used. The organic material of the sewage is quite completely oxidized by passage through the soil, and the water leaving by the under drain is of at least as high a quality as the streams into which it flows. It is not considered advisable to raise vegetables for human consumption upon such land. Grain crops and hay are generally grown.

The fact that under appropriate conditions bacteria themselves can effect a very considerable purification of sewage has led to the use of so-called septic tanks for sewage disposal. A septic tank is a closed chamber or cistern into which sewage is allowed to flow. It is usually of sufficient size so that several hours are required for any given portion to pass completely through (usually from twelve to twenty-four hours). The solid particles settle to the bottom of the tank. Aërobic bacteria quickly utilize all the free oxygen that may be dissolved in the sewage when it reaches the septic tank. Under these conditions certain of the anaërobic and facultative putrefactive bacteria bring about rapid solution of the solid organic materials. If the sewage is allowed to remain in the tank for too long a period, this decomposition goes on to the production of malodorous compounds, but when the flow is properly regulated, the action upon the constituents of the sewage within the tank is essentially digestive. The cellulose

of paper, other carbohydrates, proteins, etc., are thus converted into soluble compounds. In some cases the effluent is allowed to empty directly into a stream, but in most cases further purification is necessary. This is accomplished by means of some form of apparatus that will allow for rapid oxidation. The type first used was the sand filter. A filter bed is constructed by covering drain tile with crushed rock, gravel, and sand. The sewage from the septic tank is allowed to pass into a smaller chamber, termed a dosing chamber, which fills gradually and then empties quickly by means of automatic siphons out upon the surface of this sand filter. It has been found in practice that the sand grains become coated with a layer of bacteria, usually more or less gelatinous. Air is allowed to penetrate the sand so that the bacteria present under these aërobic conditions rapidly oxidize practically all of the organic material still remaining in the sewage. The action may be compared to that which occurs in the quick vinegar process, where an alcoholic solution is allowed to trickle into the top of a cask over beech shavings covered with acetic acid bacteria, and reappears as vinegar at the bottom of the cask. The organic materials are probably held by these organisms and by the sand particles by adsorption. The nitrogenous compounds are converted first into nitrites and then into nitrates. Sulphur appears in the effluent of the bed in combination as sulphates. Carbon is converted into carbon dioxide, and hydrogen is oxidized to water. The effluent from a filter bed which is working properly contains relatively few bacteria and little dissolved organic matter. Raw sewage as it enters the septic tank contains a considerable percentage of organic matter and from several hundred thousand to as many million bacteria per cubic centimeter. The number of bacteria does not decrease materially in passage through the septic tank. The organic material is largely rendered soluble and broken down into simple compounds. In passage through the filter beds the numbers of bacteria are very greatly decreased; sometimes in an efficient filter bed they will be below one hundred per cubic centimeter, the percentage of bacterial elimination thus being considerably over 99 per cent. At the same time the organic material is almost completely oxidized. Sewage treated in

this manner can be passed into a stream of water without fear of its creating a nuisance because of the development of odors or by endangering health.

Contact beds are modifications of sand filters. They consist of chambers or beds filled with crushed rock, slate, cinders, or coke. The sewage from the dosing chamber is allowed to run rapidly into one of these beds until it is entirely filled. It remains in contact with the material in the bed for a short time and is automatically dumped. Air, of course, follows the sewage down and comes in contact with the surfaces of all portions of the bed. The sewage as it passes through the bed is thoroughly aërated. Oxidation proceeds rapidly, and the sewage when emptied into a stream will usually not putrefy because of the thorough aëration which it has received. It is evident that this method does not result in any considerable diminution of the number of bacteria present in the sewage.

A trickling filter is essentially a modification of a contact bed. It consists of a bed or vat filled with crushed stone or cinders on which the sewage from a septic tank is spread by means of a sprinkling device. The liquid passes down over the surface of the crushed rock, etc., and the dissolved organic matter is oxidized in the same manner as has been described. The effluent of the bed is left constantly open so that it never fills with sewage. The oxidation in such a bed is extremely rapid, and the sewage of the effluent will not develop disagreeable odors when allowed to flow into a stream.

Sewage, more particularly the effluent of septic tanks, is sometimes purified by the addition of disinfectants. Those most commonly used are chlorine and the hypochlorites.

Purification of Water for Domestic Use. — A water secured from a considerable depth in the ground is usually free from sewage contamination, but large cities are rarely located under such conditions that a supply of this kind is possible. Most of these large cities secure their water supply from a lake or stream. Some cities have solved the problem by using a water which has been gathered on an uninhabited and protected watershed. Many of our western cities, for example, secure water from mountain lakes

or streams. New York City has created a great system of reservoirs, and is carefully protecting them from pollution. Chicago secures its water supply from Lake Michigan.

Many cities partially purify their water by holding it for a time in large reservoirs before use. The factors already discussed under the natural purification of water, such as sedimentation, antibiosis, etc., are active, and if sufficient time is allowed, these efficiently dispose of most of the organisms present. This method is frequently rendered more efficient by the use of coagulants which carry down in the coagulum all silt and most of the bacteria which are present.

Impure water supplies are in some cases more or less completely sterilized by the use of disinfectants. The most important of these are chlorine, the hypochlorites and ozone. It has been found that the addition of chlorine or calcium hypochlorite to water quite effectually destroys all bacteria which are present, while the free chlorine rapidly disappears, and the water does not seem to be seriously injured for domestic purposes by the time it passes through the distributing pipes. Water is purified for use in a few European cities by being exposed in thin sheets to a current of air which has been heavily charged with ozone developed by electricity. This quite efficiently sterilizes it.

In recent years some use has been made of ultra-violet light for the purification of contaminated water. The Cooper-Hewitt mercury vapor lamp gives off a very large proportion of these rays. If water is allowed to pass in a thin sheet over such a lamp, all pathogenic bacteria are rapidly destroyed.

The most common method of purification of water by cities is by the use of sand filters. These consist of layers of sand, usually a foot or more in thickness, through which the water is allowed to pass by gravity and collect in underground drains. It is then pumped to the city mains. These filters are not very efficient when first constructed, usually several days or a week elapsing before the maximum decrease in the number of bacteria in the filtered water is obtained. A filter of this kind owes its efficiency, in part at least, to the development on the surface of the sand grains of certain organisms which form a more or less gelatinous

film and prevent the passage of pathogenic organisms and eventually destroy them. The upper layer of such a filter must occasionally be removed on account of clogging, and a new layer of sand substituted. The water from such a filter should not be used for a few days after this replacement in order to give the bed an opportunity to reach its maximum efficiency. The usefulness of properly constructed filter beds of this type has frequently been demonstrated by the reduction of the death rate in cities where such a system is used.

Filters constructed of unglazed porcelain are not uncommonly used in the home and in the laboratory for filtering impure water. Some of these are constructed to fit directly upon the tap. Such filters, if properly made, are efficient in the beginning, the water coming from them being sterile, but after a time bacteria seem to grow through the pores of these filters and are then to be found in considerable numbers in the water which passes through, frequently in greater numbers than are present in the original supply. It is evident, therefore, that such filters if used at all must be thoroughly cleaned and heated to red heat at short intervals if they are to retain their efficiency.

When it is impracticable to purify water for domestic use by any of the means described above, all microörganisms may be destroyed certainly by boiling. The temperature of boiling water is sufficient to kill any of the bacteria pathogenic for man which gain entrance to the body through the alimentary tract.

CHAPTER XLIX

AIR CONTAMINATION AND EXAMINATION

It is evident that bacteria cannot under ordinary conditions grow and multiply in the air. In consequence there cannot be said to be a normal air flora. Nevertheless organisms are quite commonly present in the air, and these in the majority of cases belong to a relatively small number of species. Any organism commonly present in the air must be able to withstand a considerable amount of desiccation and grow under such conditions in nature as to be readily accessible to air currents.

Microörganisms commonly Present in Air. — All three of the groups of microörganisms which have been considered, bacteria, yeasts, and molds, are commonly present in the air.

Among the bacteria certain species of cocci are probably the most abundant. The chief of these are *Sarcina lutea* and *Sarcina aurantiaca*. *Staphylococcus aureus* and *Staphylococcus albus* are not uncommonly present in the air of dwellings. Bacilli of the hay bacillus or *Bacillus subtilis* group are ubiquitous. The spores of these organisms are exceptionally resistant. They occur in great numbers in surface soil, and find their way, in consequence, with dust into the air. The most common of these species are *Bacillus subtilis*, *B. mycoides*, and *B. vulgatus*.

Yeasts are less common in the air than are bacteria. True yeasts are sometimes found, but usually the organisms belong to the group of pseudo-yeasts or torulæ. Plates of agar or gelatin exposed to the air frequently show reddish or black colonies made up of the so-called pink or black yeasts.

The spores of certain species of molds are likewise ubiquitous. Those forms which send up erect conidiophores or sporangiophores and produce dry spores or conidia in considerable numbers

at a distance from the moist substratum are most common. The genera usually found are Penicillium, Alternaria, Aspergillus, Rhizopus, and Mucor.

How Organisms enter the Air. — Mold spores are readily detached by slight currents of air and float free, usually unattached to any particles of dust. Bacteria and yeasts, on the other hand, grow only in close contact with a moist substratum, and as long as they are growing, are not detached even by violent air currents. When the substratum dries, however, and is pulverized, the bacterial and yeast cells are floated about by currents of air, usually attached to dust particles. They may gain entrance to the air in drops of water as from the bursting of a bubble or with the droplets thrown off in sneezing or coughing. In these cases if they remain long in the air, the water droplet evaporates, leaving the organism floating free. Microörganisms are rarely detached, therefore, from moist surfaces, and dry air usually contains more bacteria than does moist air.

All microörganisms gradually subside in air which is kept motionless. The rate of subsidence depends upon the relative area of the particle, its specific gravity, the relative humidity of the air, and the velocity of air currents. An air current having a velocity of only a few centimeters per second will sustain many organisms indefinitely.

Most bacteria and yeasts are probably destroyed by desiccation and by the action of the sunlight before they have been suspended for a long period in the air. Only those organisms which are exceptionally resistant to these conditions will survive.

Transportation of Disease-producing Organisms by Air. — Contrary to general assumption, there are relatively few diseases which are readily transmitted through the medium of the air. It is probable that the tubercle bacilli when ground in sputum may exist in the air with dust, or in infectious droplets coughed out by tubercular patients. Close association with individuals having other diseases in which the organism may be thrown off in this manner may also be sufficient for transmission.

There are several popular fallacies as to the rôle of air in disease transmission. One of these, fortunately not so prevalent now as

in the past, is the so-called *night air* fallacy. It was believed at one time that breathing night air was particularly apt to produce fevers of different kinds. We know now that the origin of this belief was probably through the prevalence of mosquitoes at night. Mosquitoes transmit malaria, yellow fever, and possibly some other diseases. The disease transmission was associated in the mind with the night rather than with the insect carrier. It was also a common belief that the *air of swamps* or of regions where there is a considerable amount of decaying organic matter is unhealthful. This has been repeatedly proved to be false. Mosquitoes, and possibly other insects capable of transmitting disease, breed in swamps, and swampy districts therefore are apt to be malarial districts. The air itself has nothing whatever to do with the transmission of the disease. The odor of decaying materials cannot be held to be responsible for disease transmission, even though this odor is very decided. Flies breed in garbage and may act as carriers of disease-producing bacteria, but the air is not responsible. It was once popularly supposed also that the air found *in sewers* was capable of transmitting disease, and the various precautions used in plumbing are for the purpose of preventing any air or gas from sewers getting into dwellings. Careful examination of the air from sewers has shown that it contains relatively fewer bacteria than the air in the street or that in a dwelling. In fact, it is frequently sterile. There is no evidence that infectious diseases are ever produced by breathing sewer air. Occasionally leaky gas mains may allow the accumulation of illuminating gas in sewers. When this escapes into dwellings it may produce gas poisoning but not any infectious disease.

Numbers of Microörganisms in the Air. — *Method of Determination.* — The methods used in the determination of microörganisms in the air may be divided into two classes, those which are relative and those which are more or less absolute. In the former, it is sufficient to expose petri dishes containing nutrient agar or gelatin for equal lengths of time to the air in the various places to be compared. The microörganisms which fall upon the surface of the medium develop, and the number of colonies may be counted at the end of forty-eight hours. This of course does not give the absolute

number of bacteria in the air, but simply the relative number which will fall upon a given area in a given length of time. It is also a convenient method for securing pure cultures of the principal organisms occurring in the air.

When it is necessary to determine the exact number of microorganisms present in a given volume of air, somewhat more complicated methods are necessary. By means of an aspirator a given volume of air is drawn through a tube partially filled with sterile sugar, sand, or some similar granular substance. The sugar is then dissolved or the sand washed in a given amount of water, and the number of bacteria determined per cubic centimeter in the same manner as has been described in water analysis. After incubation the number of colonies may be counted and the number of bacteria present in a given volume of air readily calculated.

Number of Organisms in Air. — The air of some localities is practically devoid of bacteria. This has been shown to be true of the air at high altitudes, as upon mountain peaks, in the Arctic regions, and over the ocean at considerable distances from land. The outdoor air in cities may contain a very considerable number: 3500 to 4000 were found by Ellis and Fischer in a cubic meter of air in Paris streets; 100 to 125 bacteria and molds were found in this amount of outdoor air in Boston. On a windy, dry day even larger numbers may be found in the open country away from the cities. Usually, however, the air of the city is somewhat dustier and hence contains more organisms than that of the country. The air within rooms in dwellings and workshops frequently contains even greater numbers of organisms. Numbers as high as 250,000 per cubic meter have been reported by Ellis.

Relationship between Dust and Bacteria. — Dust and microorganisms are not necessarily associated. Dust which has its origin in smoke and soot may be practically sterile; in fact, the air of smoky regions may be freer of bacteria than the air in other places because of the antiseptic or disinfecting materials, such as sulphur dioxide, contained in the smoke. Methods, however, which tend to eliminate dust will in general eliminate microorganisms from the air at the same time. When it is necessary to have the air of a room practically free from microörganisms,

it must be kept free from air currents, and the walls and the floors moist. By use of this method Tyndall found that he could wholly eliminate microörganisms from air by gradual subsidence. The organisms when they come in contact with moist surfaces stick and are not again readily dislodged. The use of water in washing surfaces is a most efficient method of ridding such surfaces of microorganisms as well as of dust, and preventing the latter gaining entrance to the air. Sweeping usually greatly increases the number of microörganisms present in the air of a room. The same is true of dusting when a dry cloth is used for that purpose.

Within recent years the vacuum cleaner has been utilized to get rid of dust without allowing it to reënter the air. These cleaners are of many types, some are efficient, and some inefficient, both from the standpoint of the actual removal of dust from surfaces and from that of preventing organisms from gaining entrance to the air. Permanent installation of vacuum cleaners whereby the dust is discharged into sewers or even into the open air are preferable to those in which the dust is accumulated in a bag, for microörganisms may find their way through the latter and the finest of the dust particles may not be retained.

Frost and Armstrong have carried out a series of experiments with vacuum cleaners of various types to determine the efficiency with which they remove dust and bacteria from the surface cleaned. They developed a satisfactory method of determining the relative number of microörganisms present upon unit areas. The method employed was to cut disks of cheesecloth the size of a petri dish, leaving projections on either side for use in withdrawing the cloth. The petri dish with the cheesecloth is sterilized and a 20 per cent solution of gelatin in water is poured upon the cloth disk. The dish is re-covered and the gelatin allowed to harden, preferably for twenty-four hours. A series of such dishes are prepared, the gelatin disk is removed by means of sterile forceps from the petri dish, inverted upon the floor or other surface to be examined, pressed firmly to bring it in contact with the surface, and removed to a flask of sterile water. This is warmed sufficiently to dissolve the gelatin, is shaken thoroughly, and agar plates poured, each containing 1 cc. of this solution. The number of colonies developing

may be easily determined on the basis of the number of microörganisms present per square centimeter. By use of a method of this kind they found that in a test made with a permanent installation from 86 to 99 per cent of the microörganisms were removed. Other machines showed efficiencies varying from 57 to over 90 per cent. It is evident, therefore, that where properly installed and where care is used to see that none of the dust reënters the air of the room, the vacuum cleaner is an efficient means of getting rid of dust and microörganisms.

CHAPTER L

MILK, ITS CONTAMINATION AND EXAMINATION

Milk is an extremely complex mixture of substances. It usually contains about 87 per cent of water and about 13 per cent of solids. Of the latter there is an average of about 4 per cent fat and 9 per cent of solids other than fat. The latter comprise of protein (nitrogenous compounds) about 3.3 per cent, of lactose about 5 per cent, and of ash about .7 per cent. The nitrogenous compounds include caseinogen and albumen. There is usually about four times as much caseinogen as all the other protein constituents of milk taken together. The composition may be summarized in the following table modified from Van Slyke.

Milk = 100	Water = 87.1					
	Solids = 12.9	Fat	= 4.0			
		Solids not Fat	= 8.9	Proteins	= 3.3	Albumin = 0.7
						Casein = 2.6
						3.3
				Milk Sugar = 4.9		
				Ash (salts) = 0.7		
				8.9		

In addition there are hydrolytic enzymes, among them galactase, a proteolytic enzyme, and oxidases. It is evident that milk is a favorable culture medium for the development of bacteria. It is ordinarily impracticable to secure milk entirely free from organisms, and the changes which they bring about may therefore in a sense be considered as normal.

Changes occurring in Normal Milk. — The changes which occur in milk may be divided into several stages: the first is the stage of bactericidal action; second, the stage of the development of lactic acid; third, the neutralization of the lactic acid; and fourth, decomposition or putrefaction. In addition to these changes which may be regarded as constituting the common or normal cycle, it is

sometimes found that milk undergoes sweet curdling or may become ropy, soapy, or colored.

The Germicidal Action of Milk. — Freshly drawn milk has been shown to have a definite power of destroying bacteria. The number of bacteria present in such milk is found to decrease for a time. The length of time which this germicidal action lasts differs with the sample of milk, with the numbers and kinds of bacteria, and with the conditions under which the milk is kept. At the end of a few hours, at most, the remaining bacteria begin to multiply rapidly. Various explanations have been given for this germicidal action. Some have argued that this reduction in numbers is only seeming, not real, that it is due to the agglutinating action of the milk upon the organisms present so that bacterial clumps instead of isolated bacteria develop into colonies and are counted when the milk is plated out. Others have maintained that the action is simply a differential one, that the bacteria which first gain entrance to milk do not all find a congenial environment for development, that the forms not favored by residence in milk die off more rapidly than the organisms which can develop in milk and increase in numbers at first. It seems probable, however, that there is a certain amount of true germicidal action on the part of the milk. Antibodies of various kinds, such as hemolysins and bacteriolysins, have been determined in the milk of animals, also in the colostrum. The blood serum of the cow, as well as most of the body fluids, contain some germicidal substances, and it is to be expected that they will be given off with the milk. In addition, milk usually contains considerable numbers of leucocytes, and these do not cease action immediately after the milk is drawn, but have been found capable of ingesting bacteria for some time. It is possible, therefore, that the germicidal property of the milk is due to both the presence of true bactericidal substances and to the activity of the phagocytes.

This bactericidal property does not long persist. When the milk is kept in a warm place, it disappears within a few hours. It is more persistent when the milk is kept cool. In no case does it lead to a complete sterilization of the milk. It seems probable that the germicidal action of milk is to a certain degree specific;

that is, some bacteria are destroyed by the bactericidal substances present, while others are not affected. This property is destroyed by heating milk above 80° C. It is evident that no dependence can be put upon the bactericidal action of milk for the destruction of bacteria. As stated by Rosenau and McCoy,[1] " it cannot take the place of cleanliness and ice, but may be taken advantage of in good dairy methods."

The Souring of Milk. — The different types of lactic acid bacteria have already been discussed in Chapter XXVI, on Lactic Acid Fermentation. These organisms develop rapidly, particularly if milk is kept in a warm place. By the time 0.4 per cent lactic acid has formed, the milk tastes decidedly sour. When the acidity reaches about .75 to .8 per cent, the milk usually curdles. The lactic acid bacteria, with the exception of the organisms belonging to the *Lactobacillus bulgaricus* group, rarely produce more than 1.25 per cent of acid.

Neutralization of the Acid. — Sour milk may be kept under anaërobic conditions for a considerable time without marked change in its composition. Whenever exposed to the air, however, certain molds, particularly *Oöspora* (*Oidium*) *lactis*, develop on the surface and utilize the lactic acid as food, oxidizing it to carbon dioxide and water. This results in the disappearance of the acid reaction. In part the acid may also be neutralized by combination with the caseinogen of the milk.

Decay or Putrefaction. — As soon as the excess of the acidity of the milk has disappeared, putrefactive bacteria of different kinds begin to develop and bring about a rapid decomposition of the milk, particularly of the caseinogen.

Unusual Changes in Milk. — Milk which is heavily inoculated with organisms of the *Bacillus subtilis* group (and some other forms) may not sour normally, the lactic acid bacteria being overgrown. These organisms produce a rennet-like enzyme which causes a coagulation or sweet curdling of the milk. Later the curd is more or less completely digested.

Organisms responsible for the development of ropy milk by

[1] Bulletin 56, Hygienic Laboratory, U. S. Public Health and Marine Hospital Service, p. 487.

the formation of gums from the carbohydrates and of mucin-like substances from the proteins have already been discussed. Bacteria have also been described which are capable of producing red, yellow, blue, and even black milk. Other organisms are known which produce undesirable flavors variously characterized as soapy and bitter milks.

How Bacteria gain Entrance to Milk. — *Initial Infection.* — The tissues of a normal healthy animal are usually free from bacteria. This is true of the tissues of the mammary gland. Bacteria are, therefore, not ordinarily present in the milk as it is being secreted. As it passes down through the milk ducts, it usually comes in contact with some bacteria. Certain forms seem to develop within the milk cistern and within the larger milk ducts. The first milk drawn from the teat, therefore, usually contains more bacteria than that which is drawn later, but the numbers are not sufficiently large to make any marked difference in the total number of bacteria present in the milk of the entire milking. Usually the milk as drawn from the udder contains less than 100 bacteria to the cubic centimeter, although in some cows which seem to be perfectly normal, there may be larger numbers.

Contamination from Hair and Skin. — Unless the animal has been carefully groomed and the hair and skin of the udder and adjacent body surfaces thoroughly moistened, there will be a continual rain of dust particles and bacteria from these surfaces into the milk. This probably constitutes one of the most important sources of microörganisms in milk. Considerable differences in the number of bacteria may be demonstrated in milk taken from animals which have been carefully cleaned and those which are dry and dirty. These organisms are largely of fecal origin.

Infection from the Air. — Dust floating in the air of the building in which the cows are milked may be a fertile source of infection of the milk. This is particularly true when dusty fodder or bedding is used. Bacteria from this source are usually of the *B. subtilis* and putrefactive types.

Infection from the Hands of the Milker. — Unless the hands of the milker are carefully cleaned, there is usually plenty of opportunity for infection of the milk from this source. From a sanitary

standpoint this is particularly objectionable, as the organisms derived from this source are more apt to be capable of producing disease in man than those which have come from the animal.

Infection from Milking Utensils. — Considerable care is required to entirely cleanse milking utensils from microörganisms. Particles of rust and imperfectly soldered joints may harbor myriads of bacteria. All milking utensils should be thoroughly scalded, preferably the entire vessel should be heated to the boiling point of water to destroy the organisms present. In many cases milking utensils contribute a larger proportion of the microörganisms than any other source. Efforts have been made to cut down the initial contamination of milk by the use of milking machines. By this means one succeeds in preventing dust from the air, skin, and hair from gaining access to the milk and preventing contamination from the hands of the milker, but the milking machine is complex, not very readily cleaned, and it is found to be particularly difficult to dislodge or destroy all the bacteria present in the tubes. In the best dairies all milking utensils are sterilized in a steam chest before using.

Infection due to Carelessness in Handling. — There frequently is ample opportunity for infection of the milk after it has been drawn from the animal. It may be allowed to stand in open cans, or unclean dippers or other dairy utensils may be thrust into it. The water used in rinsing milk vessels may be contaminated.

Factors determining the Numbers of Bacteria in Milk. — The number of bacteria present in a given sample of milk depends upon several factors, the most important of these being the *initial contamination*, the *time* which has elapsed since the milk was drawn, the *temperature* at which the milk has been held, the care with which it has been *handled*, and whether or not it has been subjected to heat as in *pasteurization*.

The factors determining the initial contamination have already been discussed. It is self-evident that in securing a high quality of milk this contamination of the milk at the time it is drawn should be kept at a minimum. The time that elapses before milk is consumed is important, as the longer it is kept, in general the greater will be the number of organisms present in it. Milk is a natural

culture medium for bacteria, and they multiply until relatively enormous numbers are found. Perhaps even more important than the time element is the temperature at which the milk is held. The acid-producing bacteria, and most of the other forms which gain access to milk, grow very slowly if at all at low temperatures. Milk should, therefore, be cooled as soon as possible after it has been drawn from the animal. Milk which has been properly drawn and cooled at once will keep for several days, in some cases even for weeks, without noticeable deterioration. Usually, however, other organisms capable of multiplication at these low temperatures are present, and the milk spoils within a few days. Milk, on the other hand, which is not quickly cooled, and which is kept at room temperatures or above, quickly sours, usually in less than twenty-four hours. The care with which the milk is handled in the milk shop and in transit is an important factor in determining bacterial numbers. Pasteurization greatly reduces the numbers of bacteria present in milk.

Transmission of Disease by Milk. — Probably the most important infections transmitted by milk are the various diarrheas and dysenteries of infants. It has been proved beyond question that a betterment in the general character of a milk supply of a community or city is generally associated with a decrease in the death rate of infants, particularly those under one year of age. The intestinal tract of the infant seems to be particularly susceptible to infection by microörganisms belonging to the enteritidis, paratyphoid, and dysentery groups. A considerable proportion of the summer complaints of infants are due to the use of milk containing such organisms. Pasteurization of the milk used for infant feeding is to be advocated wherever it is impossible to secure a milk which is certainly free from such contamination.

Typhoid fever epidemics have been traced in a considerable number of cases to infection of the milk supply. Usually such epidemics are easily recognized and differentiated from those produced by contaminated water supplies by the fact that a large proportion of the victims are children, which is not generally true of a water epidemic, and the fact that the disease is generally confined to families receiving milk from a common source. The

typhoid bacillus does not produce disease in animals, hence it gains entrance to the milk only through careless handling, through the use of contaminated water in the cleansing of vessels, or more commonly by the employment of one about the dairy or in milking who has "walking typhoid" or is a bacillus carrier.

Scarlet fever and diphtheria have also been traced to contaminated milk supplies in several cases, but infections of this type are not as common as those of typhoid. Like typhoid these diseases do not attack the cow, hence the milk is usually contaminated by some individual who has handled it.

The milk from a cow having tuberculosis may or may not contain the *Mycobacterium tuberculosis*. It has already been noted in the discussion of this disease that a small proportion only of the udders of cows having tuberculosis are infected with the disease, but that a considerable proportion of such animals are constantly passing off tubercle bacilli in the feces. In general, the milk from a herd containing tuberculous animals can be shown to contain tubercle bacilli. It is also well authenticated that the use of such tuberculous milk is the common cause of a considerable percentage of the cases of tuberculosis in children. Milk should come from herds that have been tested by tuberculin and from which all tuberculous animals have been removed. When this proves impossible, milk should be pasteurized.

Some other diseases of the cow besides tuberculosis may be transmitted to man. Such are anthrax, foot and mouth disease, and possibly undulant fever. These infections are comparatively rare, however, and need not be discussed.

Methods of Milk Analysis. — The number of organisms present in milk is usually determined by plating various dilutions of the milk on nutrient agar having a reaction of + 1 to phenolphthalein. The plates are incubated at 37° C. for 48 hours, or at 22° C. for five days. The rapidity with which the organisms multiply renders it self-evident that except in milk which has been carefully drawn and carefully cared for, relatively enormous numbers will be present. It is probable that such milk is not particularly harmful to the adult, but, as has been noted above, the feeding of such milk to infants gives rise to a large proportion of their summer complaints. Milk

which is properly drawn will not contain more than 500 to 1000 bacteria per cubic centimeter when fresh. The milk as sold in cities, where it is necessarily from 36 to 48 hours or more old before use, contains many times this number. Park, for example, has found that the milk in the milk shops in New York City even during the coldest weather averages over 300,000 per cubic centimeter; during cool weather about 1,000,000; and during hot weather over 5,000,000 per cubic centimeter. The following table compiled by Bergey [1] gives the average number of bacteria per cubic centimeter found a few years ago in the milk of various American cities: —

	Bacteria per cc.
New York	4,000,000
Boston	2,300,000
Chicago	2,350,000
Baltimore	4,000,000
Wilmington	7,000,000

Methods of determining the quality of milk by differentiating the various groups of bacteria present have not been carefully worked out. Even very good milk may contain about a million of bacteria per cubic centimeter, or even more when it begins to sour, and just before it reaches its maximum acidity the number may reach many millions. It is evident that the numbers alone are of little significance, except as they indicate the care that has been used in the milking and in delivery to the consumers.

Cows not infrequently are afflicted with mastitis, an inflammation of the udder, and milk from such animals should not be used. Mastitis is usually evidenced by the secretion of considerable quantities of pus. It has been urged by some investigators that an examination of milk for pus cells should lead to a recognition of herds in which this disease was prevalent and to the condemnation of such milk. Standards, therefore, have been evolved for the potability of milk based upon the number of leucocytes and Streptococci present. The use of these counts as an index of the healthfulness of the milk received a severe blow when it was shown that Streptococci of the type of *Streptococcus lactis* constitute one of the

[1] Bergey, D. H., *Sanitary Supervision of the Collection and Marketing of Milk*, Univ. Pa. Med. Bull. 17 : 187.

commonest of the milk-souring organisms. It has likewise been shown that the number of leucocytes present in milk is subject to such wide variations and the numbers determined are so dependent upon the exact method of determination that this method has proved of little use. It has been shown that many cows normally secrete a milk which contains more than 100,000 leucocytes per cubic centimeter without evidence of inflammation or pus production in such an udder.

Classification of Market Milk. — Market milk has been classified in certain cities into certified milk, inspected milk, pasteurized milk, and uninspected milk. The term *certified milk* originally was used to indicate a milk produced by a dairy which was regularly inspected by some health board or committee of physicians. The conditions for the production of such milk were rigidly laid down and bacterial standards for the milk were adhered to. The animals from which certified milk is secured must be free from a contagious or infectious disease; the attendants must be in good health; the stables must be sanitary, well lighted, and free from dust; the milking vessels must be sterile, and every precaution must be used to prevent the access of microörganisms to the milk. After milking it must be cooled quickly, sealed in bottles, and kept cold until delivery. In most cities where certified milk is inspected, it must not contain more than 10,000 bacteria to the cubic centimeter, in a few cities not more than 25,000 to 35,000. Such milk naturally sells at a higher price than ordinary market milk.

An *inspected milk* is one which comes from cows that are tuberculin tested, and which is drawn and cared for under sanitary conditions, but where the extreme precautions used in the production of certified milk are not carried out. Most milk sold in this country is *uninspected*, and no sanitary control is maintained over the producer or even the retailer. Some cities have established maximum standards of bacterial pollution or contamination, but relatively few have been able to enforce these.

Milk which is not certainly known to be certified or inspected should be *pasteurized*. By pasteurization is meant the heating of milk for a short period of time at a temperature considerably below the boiling point. This heating is to be followed by a rapid chilling.

It has for its object the destruction of harmful bacteria and their products. The term *pasteurized milk* is sometimes used as a trade name for milk supposedly of higher grade than common market milk. In other words, it has been incorrectly used as synonymous with clean milk or pure milk. Within the last two decades the amount of pasteurized milk consumed has been greatly increased. In many of the cities of Europe commercially pasteurized milk is more commonly used than the unpasteurized. The greater proportion of the milk used in some of the large cities of the United States is pasteurized.

Pasteurization may be carried out in the home or in a commercial establishment. In the home it is best to put the milk in stoppered bottles, heat to 60° C., and hold at this temperature for twenty minutes, then cool rapidly. Very frequently home pasteurized milk is heated to such a high temperature as to impart a cooked taste and is not cooled sufficiently rapidly. Commercially pasteurized milk results in a product which will keep for a longer period than raw milk. It is, in consequence, quite popular among dairymen for commercial reasons. Commercial pasteurizers are of two types, those which pasteurize by the so-called flash process and those in which pasteurization is effected by the so-called holder process. In the flash or continuous process the milk is heated to the required temperature (80–85° C.), and maintained at that temperature for thirty seconds to a minute. It is then cooled and maintained at a low temperature until distributed. In the holder process the milk is kept at the temperature required (60–65° C.) for about half an hour. It is then cooled and bottled. It has been found in practice that the holder method is the more efficient, as it destroys a larger percentage of the bacteria. Usually over 99 per cent of the bacteria present in milk are destroyed by proper pasteurization. Among those destroyed are practically all of the pathogenic forms, including the tubercle bacillus.

The exposure of milk to the temperature of pasteurization does not appreciably affect its chemical and physical properties. Care must be used to see that milk is heated in a closed vessel, otherwise a pellicle or scum will form on the surface. This pellicle is due to the drying of the proteins of the upper layer of liquid and the con-

sequent concentration of the fatty and nitrogenous constituents. It is doubtful if the enzymes present are seriously injured by the temperature of pasteurization. Some physicians object to the use of pasteurized milk for infant feeding, but at present opinion seems to be general that pasteurized milk is assimilated quite as well as the unpasteurized. Since the general adoption in many of the poorer quarters of our large cities of the plan of boiling milk for infant feeding, there is believed to have been a marked decrease in infant mortality.

The temperature at which milk must be pasteurized is of course determined by the thermal death point of the more resistant of the pathogenic organisms which may be present, and the one upon which attention has been particularly concentrated is *Bact. tuberculosis*. The temperature of 60° for thirty minutes will quite certainly destroy this organism when pasteurization is properly carried out. Rosenau [1] draws the following conclusion. "So far as we may conclude from the evidence at hand, the heating of milk to 60° C. for twenty minutes destroys pathogenic microorganisms without seriously affecting its composition or quality and without sensibly hurting its food value. We have authority for the statement that milk pasteurized at 60° C. for twenty minutes is 'live' milk, rich in zymogens, and that such milk retains entirely the taste of fresh milk and is quite as digestible."

It has been urged that pasteurization of milk should not be advocated, inasmuch as it enables the dealer to keep dirty milk and to market it when, if it had not been pasteurized, it would have become unfit for use and easily recognized. The advantages of pasteurization, however, so far outweigh the disadvantages that it seems destined to come into very general use.

[1] M. J. Rosenau, Bulletin 56, Hygienic Laboratory, U. S. Public Health and Marine Hospital Service, p. 651.

CHAPTER LI

FOOD (EXCEPTING MILK), ITS CONTAMINATION AND EXAMINATION

Food is rarely sterile. Microörganisms of the types of bacteria, yeasts, and molds are usually found, occasionally higher forms of animal life, such as the maggots of flies, trichinae, intestinal worms, etc. Inasmuch as microörganisms are so constantly present in food, at least in the raw materials, their mere presence cannot condemn an article as unfit for human consumption. The kinds of organisms present and the extent to which they have multiplied are far more important.

Relationship of Microörganisms to Food. — Microörganisms may affect the character of food in several ways: bacteria causing infectious diseases may be present; toxins, endotoxins, and ptomaïnes may be developed by the growth of other organisms; or changes affecting the palatability or nutritive value of the food may be brought about.

Pathogenic bacteria capable of producing infectious diseases may gain entrance to food by several means. With flesh food the animal from which it came may be infected with a communicable disease, such as tuberculosis, foot and mouth disease, or the less well-defined infections due to *Bacterium enteritidis* and *Bact. paratyphosum.* Meat may be subjected to careless handling or be rendered infective by contact with a bacillus carrier, as in typhoid. Flies may carry pathogenic organisms to foods exposed for sale without being properly screened. It is possible that occasionally such organisms may be blown upon the surface with dust. Lack of cleanliness in the abattoir may permit one carcass to become infected by another or by offal or excreta. Vegetables may be infected by use of night soil as fertilizer or by being washed in

impure water. It is evident that the chance of transmission of disease to the human is least in those articles of food that are thoroughly cooked, as the pathogenic bacteria are quite certainly destroyed by exposure to the temperature of boiling water.

Organisms capable of producing poisonous toxins or endotoxins are of chief importance in foods of animal origin. The toxin of *Clostridium botulinum* is developed only in protein foods, at room temperatures, and in the tissues away from contact with free oxygen. The organism is to be regarded as a putrefactive anaerobe. This toxin has already been noted as differing from those of diphtheria and of tetanus in that it can poison when taken into the alimentary tract, but like them it is readily destroyed by heat. The *Bact. enteritidis*, on the contrary, produces a soluble endotoxin which is not readily destroyed by heat, although the bacteria themselves are killed in cooking.

Ptomaïnes are among the products of decomposition of proteins by microörganisms, many of them poisonous. They are by no means as common a cause of poisoning, however, as was supposed a decade ago. The majority of the cases of "ptomaïne poisoning" have been found to be due to specific infection by *Bact. enteritidis* and *Bact. paratyphosum*, or to the toxins or endotoxins discussed above.

Changes which affect the palatability or nutritive value of foods are frequently the result of microörganismal activity. These changes may be divided into those that are desirable and those undesirable or objectionable.

Organisms that increase palatability are of importance in a considerable number of foods. The cocoa bean undergoes a preliminary fermentation in which microörganisms play a large part before the desired flavor can be obtained in roasting. The same is true in perhaps less degree of coffee and tea. In these, enzymes of the berry and leaf are largely responsible. The flavor of cheese is in large measure due to activity of microörganisms; the same is true of butter, fermented foods, such as sauerkraut, dill pickles, and fermented beverages.

All foods, at least when exposed to contamination, sooner or later undergo changes due to microörganisms, that injure them as

food, at first affecting their palatability, and later their nutritive value.

Nitrogenous Foods of Animal Origin. — The changes produced in flesh foods during the process of ripening and subsequent decomposition have already been described, as have also those of milk and milk products.

Eggs rapidly decompose when stored at unsuitable temperatures. The initial infection of the egg with bacteria may take place while the egg is still within the oviduct of the hen, or organisms may gain entrance after the egg is laid. Egg white has been shown to possess distinct antiseptic properties. Many species of bacteria are quickly destroyed when mixed with it. This is not true of the yolk, for this is a favorable growth medium for many species of bacteria. It is not probable that this bactericidal property of egg white persists indefinitely, but it is doubtless responsible for the fact that the egg keeps as well as it does. Many species of organisms, both bacteria and molds, have been isolated from decaying eggs. Probably some of the changes that take place in stored eggs are autolytic in nature.

Fat Foods. — Fats, both of vegetable and animal origin, are not often carriers of injurious organisms. Butter may be an exception, as the tubercle bacillus may persist. The quality of the butter is determined by the quality of the milk and cream. The rancidity and gradual deterioration of some stored fats is in part due to organisms and in part to native enzymes.

Foods of Vegetable Origin. — Vegetables are most often attacked by molds, sometimes also by bacteria. These are the common causes of decay and rotting. The mold genera Penicillium, Aspergillus, and Rhizopus are particularly common. Specific disease-producing bacteria may gain entrance through use of night soil as a fertilizer in gardens as is the custom of the Chinese, or through exposure to dust and insects in the markets. Fruits may be infected through exposure or handling. They usually mold, the acidity preventing the growth of bacteria. The genus Penicillium is perhaps the most common cause, producing the green mold on oranges, lemons, apples, etc. Rhizopus and Mucor are common upon the banana and small fruits or berries. Bacterial decay also

occurs in non-acid fruits such as the banana. Raw food materials produced from the cereals are usually heavily infected with bacteria and molds. When not sufficiently dry, these develop, producing undesirable flavors and a marked change in the consistency. After baking into bread, etc., either bacteria or molds may produce change. The slimy bread organism has already been discussed. Penicillium and Rhizopus are the characteristic bread molds, the former green or yellow, the latter black.

APPENDIX

APPENDIX

KEY TO ORDERS, FAMILIES AND GENERA OF COMMON MOLDS

The following illustrated key should enable the student to determine the generic name of almost any of the molds commonly encountered in bacteriological work. It is not complete; not more than half the total mold genera recognized are included.

a. Vegetative mycelium usually without cross-walls or septa.....Order I. **Mucorales** (Page 502)

b. Spores produced in spore cases or sporangia.....Family I. **Mucoraceæ** (Page 502)

bb. Spores not produced in spore cases or sporangia.....Family II. **Chaetocladiaceæ** (Page 504)

aa. Vegetative mycelium with numerous cross walls or septa. Spores never borne in a spore case or sporangium.....Order II. **Moniliales** (Page 504)

b. Conidiophores separated, not united into definite bodies

c. Neither hyphæ nor conidia dark or smoky in color, usually clear, sometimes bright colored.....Family III. **Moniliaceæ** (Page 504)

cc. Either hyphæ or conidia dark or smoky in color, frequently both.....Family IV. **Dematiaceæ** (Page 510)

bb. Conidiophores united into definite bodies

c. Conidiophores compactly united into a globose or cylindric body, often stalked (a synnema)...Family V. **Stilbaceæ** (Page 514)

cc. Conidiophores compactly united to form a more or less spherical, unstalked body (a sporodochium).....Family VI. **Tuberculariaceæ** (Page 516)

ORDER I. MUCORALES

Family I. Mucoraceæ

a. Sporangium with a uniform wall, not thickened, usually easily broken
b. Sporangiophores arising from stolons (runners)
c. Sporangiophores borne at the nodes of the stolons. Sporangia spherical . . . . . *Rhizopus* (Fig. 181)
cc. Sporangiophores produced from the internodes of the stolons Sporangia pear-shaped . . . . . *Absidia* (Fig. 182)
bb. Sporangiophores not arising from stolons
c. Sporangia all of one kind
d. Columellæ present
e. Aerial mycelium not brown and spiny
f. Zygospores usually rare or absent, not on special hyphæ, gametes equal . . . . . *Mucor* (Fig. 183)
ff. Zygospores usually relatively abundant, on special branched hyphæ, gametes unequal . . . . . *Zygorhynchus* (Fig. 184)
ee. Aerial mycelium brown and thorny . . . . . *Spinellus* (Fig. 185)
dd. Columella absent . . . . . *Mortierella* (Fig. 186)
cc. Sporangia of two kinds or sizes
d. Large or primary sporangia with columellæ, secondary without . . . . . *Thamnidium* (Fig. 187)
dd. Both primary and secondary sporangia with columella . . . . . *Dicranophora* (Fig. 188)

FIG. 181. — Rhizopus. *a*, sporangiophores, sporangia; *b*, rhizoids; *c*, stolon; *d*, spores; *e*, columella and apophysis.

FIG. 182. — Absidia. *a*, sporangiophores, sporangia; *b*, rhizoids; *c*, stolon; *d*, spores; *e*, sporangium, columella. (Adapted from van Tieghem.)

FIG. 183. — Mucor. *a*, sporangium, with columella and sporangiophore; *b*, spores; *c*, *d*, branched sporangiophores; *e*, simple sporangiophore; *f*, chlamydospores; *g*, zygospore.

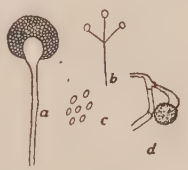


FIG. 184. — Zygorhynchus. *a*, sporangium with columella and sporangiophore; *b*, branched sporangiophore; *c*, spores; *d*, zygospore.

FIG. 185. — Spinellus. *a*, sporangium, sporangiophore; *b*, spores; *c*, aërial spiny hyphæ. (Adapted from Schröter.)

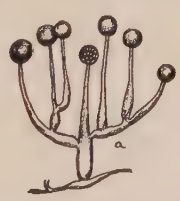


FIG. 186. — Mortierella. *a*, branched sporangiophore and sporangia. (Adapted from van Tieghem and Monnier.)

FIG. 187. — Thamnidium. *a*, branched sporangiophore with primary and secondary sporangia; *b*, secondary sporangia; *c*, spores; *d*, primary sporangium with columella; *e*, zygospore.

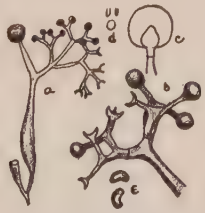


FIG. 188. — Dicranophora. *a*, branched sporangiophore with primary and secondary sporangia; *b*, secondary sporangia; *c*, primary sporangium with columella; *d*, spores, terminal sporangium; *e*, spores, secondary sporangia. (Adapted from Schröter.)

aa. Sporangium with a thickened wall, at least at apex
b. Lower portion of the sporangiophore swollen with thin wall, apical portion thick-walled. . . . . *Pilaira* (Fig. 189)
bb. Sporangium thick-walled, glutinous; sporangiophore swollen below sporangium, breaking transversely at maturity and "shooting" the sporangium away. . . . . *Pilobolus* (Fig. 190)

Family II. Chaetocladiaceæ

a. Conidia not produced in chains, produced from spherical enlargements of the tips of the branched conidiophores. . . . . *Cunninghamella* (Fig. 191)
aa. Conidia produced in chains from enlarged tips of dichotomously branched conidiophores. Parasitic on other molds. . . . . *Piptocephalis* (Fig. 192)

ORDER II. MONILIALES

Family III. Moniliaceæ

a. Conidia one celled
b. Conidiophores very short, or little different from the conidia
c. Conidiophores short, branched rarely if at all. . . . . *Oöspora* (Fig. 193)
cc. Conidiophores longer, distinctly branched. *Monilia* (Fig. 194)
bb. Conidiophores sharply differentiated and distinct
c. Conidiophores unbranched, or little branched, except at tip sometimes swollen. Conidia often in heads
d. Conidia not in chains
e. Conidiophores unbranched
f. Conidia in a head, embedded in slime. . . . . *Hyalopus* (Fig. 195)
ff. Conidia not embedded in slime. . . . . *Cephalosporium* (Fig. 196)

FIG. 189. — Pilaira. *a*, spores; *b*, sporangium with enlarged terminal portion of sporangiphore at *c*. (Adapted from van Tieghem.)

FIG. 190. — Pilobolus. *a*, sporangium and sporangiophore; *b*, portion of the bulbous mycelium. (Adapted from Zopf.)

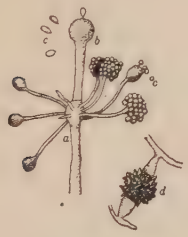


FIG. 191. — Cunninghamella. *a*, conidiophore; *b*, primary head with spores; *c*, secondary head; *d*, zygospore. (Adapted from Blakeslee.)

FIG. 192. — Piptocephalis. *a*, conidiophore and conidial heads; *b*, conidial head; *c*, haustoria penetrating mycelium of Mucor. (Adapted from Brefeld.)

FIG. 193. — Oöspora. Mycelium breaking up into chains of conidia. (Adapted from Saccardo.)

FIG. 194. — Monilia. Conidiophores and conidia. (Adapted from Went.)

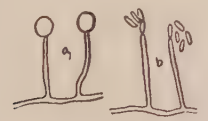


FIG. 195. — Hyalopus. *a*, conidiophores and conidial heads, the latter in mucus; *b*, conidia and conidiophores devoid of mucus. (Adapted from Nypels.)

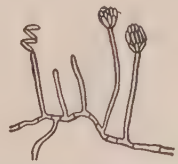


FIG. 196. — Cephalosporium. Mycelial thread with conidiophores and conidia. (Adapted from Lindau.)

ee. Conidiophores branched, pointed, each bearing a head . . . . . *Trichoderma* (Fig. 197)

dd. Conidia in chains

e. Conidiophores distinctly enlarged at tip

f. Sterigmata relatively short, numerous . . . . . *Aspergillus* (Fig. 198)

ff. Sterigmata relatively long, fewer . . . . . *Citromyces* (Fig. 199)

ee. Conidiophores not swollen at tip

f. Conidiophores branched at tip only

g. Branches equal, and radiating . . . . . *Amblyosporium* (Fig. 200)

gg. Branches more or less unequal, and not radiating

h. Conidia not embedded in slime . . . . . *Penicillium* (Fig. 201)

hh. Conidia embedded in slime . . . . . *Gliocladium* (Fig. 202)

ff. Conidiophores freely branched . . . . . *Spicaria* (Fig. 203)

cc. Conidiophores more or less profusely branched

d. Branching of conidiophores not in whorls

e. Conidia smooth

f. Conidiophores bent (decumbent) . . . . . *Sporotrichum* (Fig. 204)

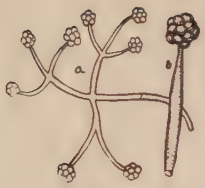


FIG. 197.—Trichoderma. *a*, branched conidiophores and conidial heads; *b*, single head of conidia. (Adapted from Harz.)

FIG. 198.—Aspergillus. *a*, conidiophore and head of conidia; *b*, conidia; *c*, detail of head.

FIG. 199.—Citromyces. Conidiophores and conidia. (Adapted from Wehmer.)

FIG. 200.—Amblyosporium. *a*, conidiophores and conidia. (Adapted from Harz.)

FIG. 201.—Penicillium. Conidiophore and conidia. (Adapted from Brefeld.)

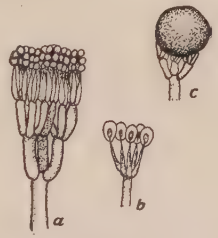


FIG. 202.—Gliocladium. *a*, conidiophores with conidia; *b*, conidia each in globule of mucus; *c*, mucus mass resulting from fusion of independent globules.

FIG. 203.—Spicaria. *a*, sterigma with conidia; *b*, conidiophore with conidia. (Adapted from Oudemans.)

FIG. 204.—Sporotrichum. Conidiophore and conidia. (Adapted from Harz.)

ff. Conidiophores erect
g. Conidia single.....*Monosporium* (Fig. 205)
gg. Conidia in heads.....*Botrytis* (Fig. 206)
ee. Conidia not smooth, warted.*Sepedonium* (Fig. 207)
dd. Branched conidiophores in whorls
e. Conidia-bearing branches short and flask-shaped....
.....*Pachybasium* (Fig. 208)
ee. Conidiophores not short and flask-shaped
f. Conidia not embedded in mucus
g. Conidia globose to ovoid.....
.....*Verticillium* (Fig. 209)
gg. Conidia cylindric to elongate.....
.....*Acrocylindrium* (Fig. 210)
ff. Conidia inclosed in mucus to form heads.....
.....*Acrostalagmus* (Fig. 211)
ccc. Conidia borne on differentiated intercalary cells of the conidiophore.....
.....*Nematogonium* (Fig. 212)

FIG. 205. — Monosporium. Conidiophore and conidia. (Adapted from Saccardo.)

FIG. 206. — Botrytis. Conidiophores and conidia. (Adapted from Frank.)

FIG. 207. — Sepedonium. Conidiophores and conidia. (Adapted from Tulasne.)

FIG. 208. — Pachybasium. Conidiophore and conidia. (Adapted from Oudemans.)

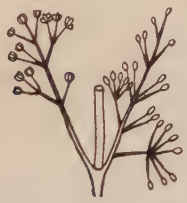


FIG. 209. — Verticillium. Conidiophore and conidia. (Adapted from Harz.)

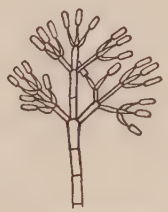


FIG. 210. — Acrocylindrium. Conidiophores and conidia. (Original.)

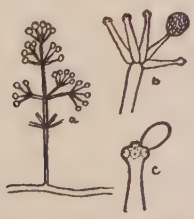


FIG. 211. — Acrostalagmus. *a*, conidiophore and conidia; *b*, tip of conidiophore; *c*, attachment of the conidia. (Adapted from Corda.)

FIG. 212. — Nematogonium. Conidiophores and conidia. (Adapted from Saccardo.)

aa. Conidia more than one-celled
b. Conidia two-celled
c. Conidia lateral on conidiophores
d. Single, not clustered.....*Mycogone* (Fig. 213)
dd. Clustered on swollen cells of conidiophore.....
.....*Arthrobotrys* (Fig. 214)
cc. Conidia terminal.....*Trichothecium* (Fig. 215)
bb. Conidia more than two-celled, elongate..*Ramularia* (Fig. 216)

Family IV. Dematiaceæ

a. Conidia one-celled
b. Conidia dark colored
c. Conidia not in chains
d. Conidia in terminal heads
e. Conidiophores not branched
f. Apex with sterigmata...*Stachybotrys* (Fig. 217)
ff. Apex without sterigmata..*Periconia* (Fig. 218)
ee. Conidiophores branched
f. Conidiophores forked below apex; conidia oblong.....*Synsporium* (Fig. 219)
ff. Conidia inserted irregularly on jar-like basidia
.....*Basisporium* (Fig. 220)

FIG. 213. — Mycogone. Conidiophores and conidia. (Adapted from Saccardo.)

FIG. 215. — Trichothecium. Conidiophores and conidia. (Adapted from Matruchot.)

FIG. 217. — Stachybotrys. Conidiophores and conidia. (Adapted from Oudemans.)

FIG. 219. — Synsporium. Conidiophore and conidia. (Adapted from Cavara.)

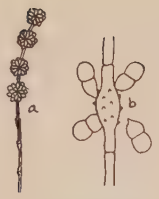


FIG. 214. — Arthrobotrys. *a*, conidiophores showing arrangement of the conidia; *b*, detail of the same. (Adapted from Corda.)

FIG. 216. — Ramularia. Conidiophores and conidia. (Adapted from Lindau.)

FIG. 218. — Periconia. *a*, conidiophore with head of conidia; *b*, detail of spore attachment. (Adapted from Saccardo.)

FIG. 220. — Basisporium. Conidiophores and conidia. (Adapted from Abbott.)

dd. Conidia single and terminal on unbranched conidiophores. . . . . *Acremoniella* (Fig. 221)

cc. Conidia in chains

d. Conidiophores unbranched, conidial chains lateral. . . . . *Dematium* (Fig. 222)

dd. Conidiophores branched. . . . *Hormodendrum* (Fig. 223)

bb. Conidia light-colored, conidiophores dark. . . . . *Mesobotrys* (Fig. 224)

aa. Conidia more than one-celled

b. Conidia two-celled

c. Conidiophores very short, little differentiated. . . . . *Dicoccum* (Fig. 225)

cc. Conidiophores distinct, usually erect

d. Conidia terminal and lateral on much branched conidiophore. . . . . *Cladosporium* (Fig. 226)

dd. Conidia terminal on thread-like sterigmata. . . . . *Scolecobasidium* (Fig. 227)

bb. Conidia more than two-celled

c. Conidia with cross septa only

d. Conidia formed singly, terminal or lateral. . . . . *Helminthosporium* (Fig. 228)

FIG 221. — Acremoniella. Conidiophores and conidia. (Adapted from Marchal.)

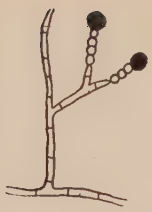


FIG. 222. — Dematium. Conidiophore and conidia. (Adapted from Saccardo.)

FIG. 223. — Hormodendrum. Conidiophores and conidia. (Adapted from Bruhne.)

FIG. 224. — Mesobotrys. Conidiophores and conidia. (Adapted from Abbott.)

FIG. 226. — Cladosporium. Conidiophores and conidia. (Adapted from Janczewski.)

FIG. 225. — Dicoccum. Conidiophore and conidia. (Adapted from Harz.)

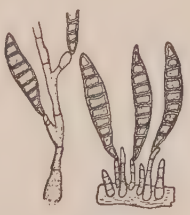


FIG. 228. — Helminthosporium. Conidiophores and conidia. (Adapted from Saccardo.)

FIG. 227. — Scolecobasidium. (Adapted from Abbott.)

dd. Conidia produced in terminal heads or lateral whorls....
e. In lateral whorls.....*Spondylocladium* (Fig. 229)
ee. In terminal heads.....*Acrothecium* (Fig. 230)
cc. Conidia with septa both cross and longitudinal
d. Conidia solitary and apical
e. Conidia cross shaped, rough.....
.....*Tetracoccosporium* (Fig. 231)
ee. Conidia smooth
f. Conidiophores bent down (decumbent).....
.....*Stemphylium* (Fig. 232)
ff. Conidiophores erect...*Macrosporium* (Fig. 233)
dd. Conidia not solitary
e. Conidia in heads.....*Dactylosporium* (Fig. 234)
ee. Conidia in chains.....*Alternaria* (Fig. 235)

Family V. Stilbaceæ

a. Hyphæ, coremium and conidia hyaline or light colored
b. Coremium with spores in a head at top, also lateral heads.....
.....*Tilachlidium* (Fig. 236)

FIG. 229. — Spondylocladium. Conidiophores and conidia. (Adapted from Preuss.)

FIG. 231. — Tetracoccosporium. Conidiophores and conidia. (After v. Szabó.)

FIG. 233. — Macrosporium. Conidiophores and conidia. (Adapted from Berlese.)

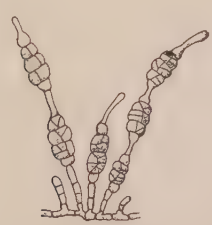


FIG. 235. — Alternaria. Chains of conidia. (After Saccardo.)

FIG. 230. — Acrothecium. Conidiophore and conidia. (Adapted from Saccardo.)

FIG. 232. — Stemphylium. Conidiophores and conidia. (Adapted from Saccardo.)

FIG. 234. — Dactylosporium. Conidiophores and conidia. (Adapted from Corda.)

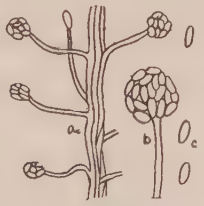


FIG. 236. — Tilachlidium. *a*, coremium and separate conidiophores with heads of conidia.; *b*, head of conidia; *c*, conidia. (Adapted from Oudemans.)

bb. Coremium with conidia on sides as well as top . . *Isaria* (Fig. 237)
aa. Hyphæ, coremium and spores dark. Conidia in chains . . . . . .
. . . . . *Stysanus* (Fig. 238)

Family VI. Tuberculariaceæ

a. Conidia and hyphæ hyaline or light colored
b. Conidia one-celled
c. Sporodochium without spines . . . . . *Hymenula* (Fig. 239)
cc. Sporodochium with marginal hairs or spines . . . . . .
. . . . . *Volutella* (Fig. 240)
bb. Conidia several-celled, sickle-shaped . . . . . *Fusarium* (Fig. 241)
aa. Conidia or hyphæ dark or smoky
b. Conidia one-celled . . . . . *Epicoccum* (Fig. 242)
bb. Conidia several-celled . . . . . *Thyrococcum* (Fig. 243)

FIG. 237. — Isaria. *a*, Coremia; *b*, single conidiophores. (Adapted from Saccardo.)

FIG. 238. — Stysanus. *a*, coremium; *b*, conidiophore with chain of conidia. (Adapted from Lindau.)

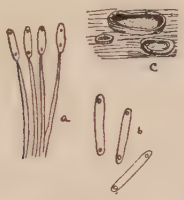


FIG. 239. — Hymenula. *a*, conidiophores; *b*, conidia; *c*, sporodochia. (Adapted from Boudier.)

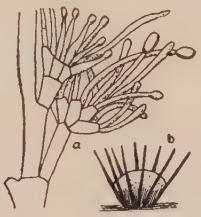


FIG. 240. — Volutella. *a*, detail of conidiophores and conidia; *b*, sporodochium. (Adapted from Boulanger.)

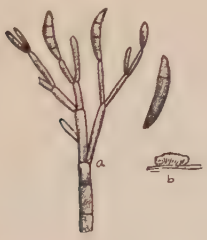


FIG. 241. — Fusarium. *a*, details of conidiophores and conidia; *b*, sporodochium. (Adapted from Saccardo.)

FIG. 242. — Epicoccum. Sporodochium and conidio. (Adapted from Penzig.)

FIG. 243. — Thyrococcum. Sporodochium and conidia. (Original.)

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